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Predicting successful outcome of singleton and multiple pregnancies after assisted reproductive technologies (ART)

Thesis presented by

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Contents

List of Tables

List of Figures and Images

List of abbreviations

APH	Antepartum haemorrhage
AMH	Anti-Müllerian hormone
AFC	Antral follicle count
array-CGH	Array-comparative-genomic hybridisation
BMI	Body mass index
HCG	Chorionic Gonadotrophin
COH	Controlled ovarian stimulation
CUMH	Cork University Maternity Hospital CUMH
CF	Cystic fibrosis
DNA	Deoxyribonucleic acid
DCDA	Dichorionic, diamniotic
DOR	Diminished ovarian reserve
DET	Double embryo transfer
EC	Egg collection
ESET	Elective single embryo transfer
FSH	Follicle stimulating hormone
FET	Frozen- embryo transfer
GnRH	Gonadotrophin- releasing hormone
HOM	Higher order multiple
HFEA	Human Fertilisation and Embryology Authority
OHSS	Hyperstimulation syndrome
IVF	In vitro fertilization
ICSI	Intracytoplasmic Sperm Injection
IUD	Intrauterine demise
IUI	Intrauterine insemination
LGA	Large for gestational age
LLETZ	Large Loop Excision of the Transformation Zone
LH	Luteinising hormone
NICU	Neonatal Intensive Care Unit
NNU	Neonatal Unit

NGS	Next generation sequencing
OR	Odds Ratio
OD	Oocyte donation
PCOS	Polycystic Ovarian Syndrome
PPH	Postpartum haemorrhage
PET	Pre-eclampsia
EPU	Pregnancy assessment unit
PIH	Pregnancy-induced hypertension
PGD	Pre-implantations genetic diagnosis
PGS	Pre-implantations genetic screening
ROS	Reactive oxygen species
ART	Assisted reproductive technologies
RDS	Respiratory distress syndrome
SGA	Small for gestational age
ASRM	American Society for Reproductive Medicine
SC	spontaneously conceived
TESE	Testicular sperm extraction
ESHRE	The European Society of Human Reproduction and Embryology
ICMART	The International Committee for Monitoring ART
3D	Three dimensional
TSH	Thyroid stimulating hormone
TTTS	Twin transfusion syndrome
2D	Two dimensional
VTS	Vanishing twin syndrome
VDR	Vitamin D receptor
WHC	Women's Health Cohort study
WHO	World Health Organization

Declaration

I certify that this thesis which I now submit for assessment for the award of Doctor of Medicine, does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Published and presented work

Articles published in a peer reviewed journal

- *The maternal and perinatal implications of hypertensive disorders of pregnancy in a multiple pregnancy cohort.*
  D Hayes- Ryan, S Meaney, A O'Mahony, **M. Geisler,** K. O'Donoghue
  Acta Obstet Gynecol Scand. 2020; 00:1–12

- *Obstetric and perinatal outcomes of twin pregnancies conceived following IVF/ICSI treatment compared with spontaneously conceived twin pregnancies.*
  Geisler ME, O'Mahony A, Meaney S, Waterstone JJ, O'Donoghue K
  Eur J Obstet Gynecol Reprod Biol. 2014 Oct; 181:78-83

- *Stress and the impact on the outcome of medically assisted reproduction.*
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- *Oocyte Donation Pregnancies- non-disclosure of oocyte recipient status to obstetric care providers and perinatal outcomes.*
  Geisler M, Meaney S, O'Donoghue K, Waterstone J
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- *Intrauterine insemination- No more Mr. N.I.C.E. guy?*
  Geisler ME, Ledwidge M, Bermingham M, McAuliffe M, McMenamin MB, Waterstone JJ. Eur J Obstet Gynecol Reprod Biol 2017 Mar; 210:342 - 347. doi: 10.1016/j.ejogrb.2017.01.016. Epub 2017 Jan 18.

- *The role of ultrasound measurements and biochemical markers for predicting pregnancy outcome in nulliparous women undergoing ART.*
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- *P - 507 Stress and the impact on assisted reproductive technology (ART) outcomes.*
  M. Geisler, S. Meaney, J.Waterstone, K. O'Donoghue
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- *A review of intrauterine deaths in twin pregnancies occurring in Cork University Maternity Hospital between 2009 and 2012.*
  L. Szittya, A.G. Morris, **M. Geisler**, K. O'Donoghue
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Presentations

- *Stress and the impact on assisted reproductive technologies.*
  Geisler ME, Meaney S, Waterstone J, O'Donoghue K
  Oral poster presentation. European Society for Human Reproduction and Embryology, Annual Conference, Vienna 2019

- *Intrauterine insemination- No more Mr. N.I.C.E. guy?*
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  First prize for best oral presentation by Irish Fertility Society
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- *Under-disclosure of egg donation pregnancies to obstetric care providers.*
  Geisler M, Meaney S, O'Donoghue K, Waterstone J
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  Irish Fertility Society Meeting 2013.

Executive Summary

Introduction

Pregnancies conceived through assisted reproductive technologies (ART) are increasing in prevalence in maternity units in Ireland due to the increasing numbers of women attending for ART. This is due in a large part to the increasing age profile of women attempting to conceive for the first time but also due to the increasing success of ART itself (1).

The advent of ART and the increasing numbers of pregnancies resulting from ART also brought an increase in the numbers of multiple pregnancies. Multiple pregnancy has been shown to carry a higher rate of preterm delivery than singleton pregnancy and a higher rate of adverse obstetric and perinatal outcomes. This led to many governing bodies, including the Human Fertilisation and Embryology Authority (HFEA) in the UK and the American Society for Reproductive Medicine (ASRM) in USA calling for a reduction in the number of embryos being transferred in order to reduce the numbers of multiple pregnancies from ART (2). Elective single embryo transfer (eSET) is advised in good prognosis patients- women under the age of 35 - 37 with more than one good quality blastocyst available for transfer (3). Although livebirth rates are lower in eSET versus double embryo transfer (DET) cycles, the risk of multiple pregnancy and its associated complications is lower. Furthermore, several studies, including a Cochrane Database System Review, have demonstrated that when cumulative livebirth rates of an unsuccessful eSET cycle followed by a subsequent frozen-thawed embryo are compared to a DET cycle they are

comparable, with a significantly reduced risk of multiple pregnancy (4). We wished to investigate if there were identifying factors which would allow a more individualised approach to selecting the number of embryos for transfer according to obstetric risk factors, particularly pre-term labour.

The original hypothesis of the thesis was that, due to the increased risk of preterm labour associated with multiple pregnancies, if a woman could be identified prior to in vitro fertilisation (IVF)/ intracytoplasmic sperm injection (IVF/ICSI) treatment as being at high risk of preterm labour then she should have a single embryo transfer. Conversely, if a woman was deemed low risk for preterm labour than it may be more reasonable to consider a double embryo transfer, allowing for a more individualised embryo transfer policy tailored to the risk profile of the woman.

Nulliparous women were selected for inclusion. Exclusion criteria were; multiparty, previous preterm delivery (spontaneous delivery prior to 37 weeks gestation) or late miscarriage (pregnancy loss between 12+1 and 23+6 weeks gestation), a history of cervical excisional surgery (Large Loop Excision of the Transformation Zone (LLETZ) or knife conisation), pre-existing serious maternal medical disorders that may increase risk of pre-term delivery (e.g. essential hypertension, poorly controlled diabetes, epilepsy), the use of confounding treatment (e.g. elective cervical suture placement). The rationale for these exclusion criteria was to ensure that there was no pre-existing risk of spontaneous (cervical excisional surgery, late miscarriage) or iatrogenic (serious maternal co-morbidities) preterm delivery amongst the recruited cohort. One of the factors studied in the recruited cohort was the volume and dimensions of the uterus immediately prior to

commencing Follicle Stimulating Hormone (FSH) stimulation in an IVF/ICSI cycle. This was performed with both 2-dimensional (2D) and 3-dimensional (3D) ultrasound to assess if these measurements would inform pre-term labour risk, particularly in twin pregnancies.

An interim assessment identified that the number of twin pregnancies occurring in the recruited cohort was too low to adequately power the prospective cohort study (detailed in Chapter Three) and adequately test our hypothesis. Furthermore, the retrospective study, detailed in Chapter Two, which examined the obstetric and perinatal outcomes of twin pregnancies conceived through IVF/ICSI and compared them to spontaneously conceived twins, demonstrated that ART conceived twins had reasonable perinatal outcomes with only a slightly higher rate of moderate preterm delivery and a very low rate of extreme prematurity. The retrospective study demonstrated that, with improvements in obstetric and perinatal care including dedicated twin clinics with appropriate ultrasound monitoring, twin pregnancies, irrespective of mode of conception have reasonably favourable outcomes. These findings were published in a peer reviewed article 'Obstetric and perinatal outcomes of twin pregnancies conceived following IVF/ICSI treatment compared with spontaneously conceived twin pregnancies' (5).

It also recognises that singleton pregnancies are a significantly lower risk pregnancy and therefore the regulation over the numbers of embryos transferred is important, in order to achieve a low rate of twin pregnancies. The elective single embryo transfer policy that was being increasingly employed by Cork Fertility Centre during the years of recruitment resulted in a lower twin pregnancy rate than

expected. In view of these findings the scope of the thesis was expanded to include singleton pregnancy outcome and to examine multiple other factors, including stress, lifestyle factors, demographics and biochemistry in the wider ART population, that may impact on treatment cycle and pregnancy outcome after IVF/ICSI.

Retrospective study (Chapter 2)

Chapter two details the retrospective study of the pregnancy outcomes of singleton and twin pregnancies conceived following ART. The twin pregnancy cohort was compared to a cohort of spontaneously conceived twins (6). Dichorionic diamniotic twins were specifically selected as these twins result from the transfer of two embryos (iatrogenic twinning) and therefore could have been avoided if a single embryo was transferred. This represents an option for intervention should certain women be identified as having an increased risk of adverse twin pregnancy outcome following ART. The study found that ART conceived twins had a high livebirth rate, of 291 ART conceived twin pregnancies 86.8% resulted in a livebirth of one or both twins. ART conceived twins had similar obstetric and perinatal outcomes to spontaneously conceived twins. There was no difference in the rates of preterm delivery between the groups and there was a very low rate of extreme prematurity that did not differ between the groups. Overall, perinatal outcomes were similar.

The outcome of singletons conceived from IVF/ICSI was also retrospectively studied. Unfortunately, it was not possible to have a matched spontaneously conceived cohort of singleton pregnancies. Overall, singleton outcomes (n = 1790)

were very favourable with a high livebirth rate (80%) and a high term delivery rate (93%).

Vanishing twin syndrome (VTS) singletons were also analysed for outcome. VTS singleton outcomes were compared with singleton outcomes. There was no significant difference in gestation at delivery between the two groups. However, VTS singletons were significantly more likely to be delivered by emergency caesarean section (24% v 15%).

Pregnancy outcomes of singleton pregnancies versus twin pregnancy were analysed. Singleton pregnancies were more likely to result in a livebirth while twin pregnancies were almost twice as likely to result in miscarriage of one or both twins. Twins were four times more likely to be delivered before 36 weeks gestation than singletons. Twins are three times more likely to be admitted to the Neonatal Unit (NNU) than singletons.

These results demonstrate that the mode of conception of a twin pregnancy does not incur a significantly higher obstetric or perinatal risk. However twin pregnancy itself has a higher rate of adverse outcomes when compared to singleton pregnancy. Furthermore, VTS singletons (pregnancies that began as twins) demonstrated a significantly higher rate of caesarean delivery. These findings further support the guidance for elective single embryo transfer.

Prospective Cohort Study (Chapter Three)

Chapter three details the findings of the prospective cohort study of nulliparous women attending Cork Fertility Centre at the outset of their IVF/ICSI cycle. The recruited women underwent both a 2D and 3D ultrasound of the uterus immediately prior to commencing FSH stimulation. Women were also invited to complete a detailed survey on lifestyle and demographics. The initial aim of the study was to analyse the outcomes of twin pregnancies conceived by IVF/ICSI, however, an interim assessment recognised that the number of twin pregnancies in the recruited cohort was too low to adequately power the study and it was therefore expanded to include singleton pregnancy outcomes. The low rate of twin pregnancy numbers was as a result of logistical issues limiting recruitment and the recent adoption of a successful eSET programme in the clinic, resulting in a lower twin pregnancy rate.

The women were prospectively followed up to analyse the occurrence of pregnancy, or not, and any factors that may influence conception rates. All ongoing pregnancies were analysed for the occurrence of obstetric complications, including gestational diabetes, pregnancy induced hypertension and pre-eclampsia.

One hundred and forty- two women were recruited for this study according to the inclusion criteria. None of the interrogated demographics or lifestyle factors (including smoking, alcohol intake and BMI) demonstrated a significant impact on the rate of conception nor on the rate of pregnancy loss. Neither uterine length nor volume impacted on pregnancy rates from IVF/ICSI or on preterm delivery rates. Overall, the rate of preterm delivery was 19% with only one very preterm delivery and no extreme preterm deliveries. Women with a higher antral follicle count had a

four-fold increased chance of livebirth compared with women with a reduced antral follicle count.

Cross-Sectional Study (Chapter Four)

Chapter four details the findings of the cross-sectional study which was a survey-based study assessing the impact of demographics, lifestyle and psychological factors on ART outcome. There is evidence in the medical literature to suggest that high perceived stress in early pregnancy is associated with increased rates of miscarriage (7).

The aim of this study was to determine if psychological stressors have an impact on IVF/ICSI treatment cycle outcomes including miscarriage rates. A survey-based study of 320 women recruited prior to commencing IVF/ICSI treatment and followed prospectively for treatment outcome (negative/positive pregnancy test, first trimester miscarriage). The survey focused on perceived stress, emotional well-being, maternal social support and outlook. The women were asked to grade their responses according to a specified scale. 290 (90%) women proceeded to ART treatment of which 58% conceived. Analysis of individual life stressors in the preceding 12 months, including job stress or serious financial problems did not reveal significance in terms of conception. A small number had experienced serious illness in the preceding 12 months which demonstrated significance in terms of not achieving pregnancy.

The women were further analysed according to livebirth and pregnancy loss (biochemical pregnancy and miscarriage). Individual life stressors revealed a significantly higher rate of pregnancy loss amongst those who reported a stressful/demanding job. The findings suggest that stressors do not impact greatly on conception rates from ART but may negatively impact on miscarriage rates. Job-related stress, in particular, is associated with higher chance of miscarriage. This suggests that there may be a role for stress management in early pregnancy.

During ART there is an element of control over the number of embryos being transferred and the resultant rate of twin pregnancy. Therefore, in the absence of any robust predictors of successful, or indeed adverse, outcome it is preferable to aim for single embryo transfer and a singleton pregnancy. Couples with two good quality embryos should be counselled regarding the increased risk of iatrogenic twinning and its implications, associated with double embryo transfer. The thesis finds a link between stress and miscarriage which may warrant future work.

Chapter 1 - Introduction

Chapter 1 - Introduction

The World Health Organisation (WHO) defines infertility as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months, or more, of unprotected sexual intercourse (8).

Approximately 1 in 7 couples will experience fertility problems during their reproductive lives. In recent years, there has been a steady increase (5 - 10% per annum) in the number of couples attending for fertility treatment. In Ireland, the age of women at the time first pregnancy is increasing, reflecting societal and economic factors resulting in women delaying childbearing until the third decade (9). The corollary of this is an increasing number of couples struggling to conceive.

There are several aetiologies of subfertility that may be attributed to the female partner, the male partner or both partners. Unexplained subfertility is the most frequently occurring aetiology (25%), where there is no identifiable cause for the couple's inability to conceive. Male factor subfertility is due to abnormalities of sperm production or ejaculation and accounts for a further 30% of aetiologies. Female factors account for another 30% and include diminished ovarian reserve, tubal factors and endometriosis (10).

Assisted reproductive technologies (ART) were first examined in the mid 1800's when artificial insemination was first performed by Dr. Marion Sims at The Women's Hospital, New York. The area of in vitro fertilisation was explored in the early 1900's, initially with Harvard scientist Gregory Pincus' work with rabbits which

was advanced by John Rock and Miriam Menkin who, in 1944, achieved in vitro fertilisation of a human oocyte by human spermatozoa.

Scientists internationally continued in attempts to perfect in vitro fertilisation, including Robert Edwards, who travelled to John Hopkin's University Hospital for a six-week fellowship, where he finally achieved successful fertilisation of a human egg. Robert Edwards then met Patrick Steptoe in London and continued in his pursuit to implant a fertilized oocyte into a female uterus. Ultimately, they become the first scientists to achieve a successful pregnancy, resulting in the first live birth of a "test tube baby" Louise Brown in 1978. Australia and USA quickly followed with live births in 1980 and 1981 respectively, heralding the beginning of IVF success internationally.

IVF and other forms of ART have continued to develop in technique and popularity with more than 350,000 babies born annually from IVF/ICSI treatment worldwide. The International Committee for Monitoring ART (ICMART) most recently reported on the triennia 2008 - 2010. During this period, there were 4,461,309 cycles initiated, resulting in the birth of approximately 1,144, 858 babies (11). Societal changes, particularly in the role of women, has resulted in and increasing number of women delaying childbearing and ultimately requiring ART to achieve a pregnancy.

Access to ART is variable with some countries, such as the UK, providing state-funded treatment options for couples. Other countries provide only very limited/no state funding for ART, requiring the couples to self-fund treatment (12, 13). In Ireland, where there is no state funding for ART (although the medication costs are

funded) ART is solely provided by private clinics with the average IVF cycle costing a couple approximately €4,000 or more.

1.1 What are assisted reproductive technologies?

Assisted reproductive technologies (ART) comprise of a range of medical treatments involving the application of laboratory or clinical technology to human gametes and/or embryos in order to achieve pregnancy in a subfertile couple.

1.1.1 Intra-uterine insemination

Intrauterine insemination (IUI) is frequently the first line treatment option for those couples with unexplained subfertility. The pre-requisite to IUI is patent Fallopian tubes and a suitably prepared sperm sample. IUI can be carried out in a natural cycle or with controlled ovarian stimulation (COH). Superovulation with gonadotrophins (Follicle Stimulating Hormone; FSH) results in the development of 1 - 2 dominant follicles, the development of which is monitored by transvaginal ultrasonography. Once the dominant follicle is at least 18mm in size ovulation is triggered with human Chorionic Gonadotrophin (hCG). Insemination of a prepared sperm sample into the uterine cavity is performed the following day. IUI/COH results in a per cycle pregnancy rate ranging between 11 - 16.4% (14-17).

IUI has the benefit of occurring within the timeframe of a natural menstrual cycle, is less invasive - requiring only catheterisation of the uterus to deposit the

sperm sample, and less costly than IVF treatment. However, the success rates are lower and there is concern regarding the higher multiple pregnancy rates associated with IUI. To this end, in 2013 the National Institute for Health and Care Excellence updated the guidance on IUI/COH, advising that IUI should no longer be offered to couples with a diagnosis of unexplained subfertility. Such couples should be advised two years of expectant management before proceeding directly to IVF (18). This guidance is based on low- to very low-quality evidence indicating that treatment with IUI (with or without COH) shows no significant increase in live birth over expectant management (19, 20). One of the concerns of the NICE guideline development group is the high rate of multiple pregnancies with IUI/COH and the lack of control over the occurrence of multiple pregnancies when compared with IVF and single embryo transfer. This is a valid concern to address given the well-documented increased perinatal risk of multiple pregnancies (21, 22). Patients who have not achieved a live birth with IUI treatment generally move on to IVF treatment which carries higher success rates but is more expensive and invasive than IUI.

1.1.2 In vitro fertilisation

In vitro fertilisation (IVF) was originally developed to overcome bilateral tubal occlusion. Today, IVF is used for a variety of aetiologies of subfertility- failure to conceive from IUI, diminished ovarian reserve, endometriosis, moderate sperm dysfunction. The number of cycles of IVF continues to increase with the Human Fertilisation and Embryology Authority showing a near 5% year on year increase in the number of IVF cycles undertaken in the UK alone (23).

Current ART practice involves ovarian superovulation with gonadotrophin - releasing hormone (GnRH) analogues, most frequently FSH alone, or in combination with luteinising hormone (LH). Once the lead follicle is at least 18mm in size and there are at least three follicles greater than 17mm ovulation is triggered with hCG or a GnRH agonist. Thirty-six hours following the administration of ovulatory medications, oocyte retrieval is performed. This typically involves transvaginal ultrasound-guided needle-aspiration of follicular fluid and retrieval of mature oocytes from this fluid. The oocytes are then inseminated with a prepared sperm sample (requiring a sperm count of > 500,000 motile sperm to be suitable for IVF) and fertilisation is awaited. Fertilisation is identified 18 - 24 hours later by the presence of two pronuclei in the fertilised oocyte.

Standard IVF requires the presence of more than 500,000 motile sperm in the total ejaculate. Intracytoplasmic sperm injection (ICSI) is adopted in situations where there is severe sperm dysfunction. In this situation, the cytoplasm of each mature oocyte is injected with a single spermatozoon and the fertilisation rate is assessed 18 hours later. ICSI may also be considered where fertilisation has failed with IVF despite normal sperm preparation.

On day 3 following oocyte retrieval the resultant embryos are assessed for grading based on their morphological appearance. Embryo transfer typically occurs on day 3 or day 5 (usually between 2 - 5 days), following oocyte retrieval. The decision for embryo transfer depends on the quantity of good-quality oocytes. Embryo quality is based on the morphological appearance of the embryo according to the developmental stage. If the numbers and quality of embryos are deemed sufficient

then embryo culture will be continued to blastocyst stage (day 5 post oocyte retrieval). At this stage, the superior quality blastocyst (s), based on morphological appearance, are selected for transfer into the uterus. Day five, or blastocyst, transfer has been shown to have a positive effect on pregnancy rates (24). Luteal phase support is typically with vaginal or parenteral progesterone and /or hCG. Pregnancy testing by detection of urinary hCG or serum βhCG is usually performed 18 days following oocyte retrieval

1.2 Recent developments in ART

1.2.1 Gamete donation

Gamete donation is most frequently sperm donation for couples where the male partner has azoospermia. More recently, sperm donation is being used by single women and homosexual women to achieve pregnancy. Donor sperm can be used for intrauterine insemination or IVF/ICSI. Oocyte donation, since its introduction 30 years ago, has become an increasingly common solution to involuntary childlessness. The oocyte donor undergoes superovulation and oocyte retrieval and the retrieved oocytes are fertilized (with IVF or ICSI) with the male partner's (or donor) sperm. Initially, it was used to enable women with premature ovarian failure to conceive (25). Increasingly, it is recommended to women of advanced reproductive age and to those with intractably poor embryo quality (26). The most recent European data from 34 countries, reported 25,187 egg donation cycles representing a 16.6% increase on the previous year and a 54% increase on the number of cycles in 2008 (27, 28).

1.2.2 Cryopreservation

Cryopreservation of both gametes and embryos has become more successful in the past 5 years since the introduction of vitrification (29, 30).

Sperm cryopreservation has been used for many years at sperm banks for donor sperm and following surgical sperm retrieval in men with oligozoospermia.

The main indication for oocyte cryopreservation is fertility preservation prior to chemotherapy or radiotherapy. Increasingly, oocyte cryopreservation is now being used by women for social reasons, i.e. fertility preservation until socially the time is right to pursue pregnancy e.g. after establishment of her career or delay in meeting a suitable male partner. Oocyte cryopreservation has limitations in terms of safeguarding fertility (31). The most recent report from the Human Fertilisation and Embryology Authority (HFEA) in the UK for the first time contained data on oocyte cryopreservation (23). It reported a 25 - 30% year on year rise in the number of women undertaking oocyte cryopreservation, with 102 cycles of IVF in 2013 (rising to 129 cycles in 2014) using cryopreserved oocytes, yielding only a 14% pregnancy rate (compared with an average 26% success rate for IVF with fresh oocytes).

Embryo cryopreservation is becoming increasingly successful due to the more widespread use of a recent cryopreservation method known as vitrification. There is some evidence that a frozen - embryo transfer (FET) may result in improved pregnancy rates when compared to a fresh embryo transfer (31).

Vitrification involves an extremely high cooling rate (15,000 ^{o}C to 30,000 ^{o}C/minute). High concentrations of cryoprotectant are used to prevent ice crystal

formation. This rapidly dehydrates the cells and rapid submersion into liquid nitrogen avoids the development of the large intracellular or extracellular ice crystals that can be damaging to the cells during the freeze/thaw process. Slow freezing is more time-consuming and less effective at avoiding ice crystal formation due to the use of a lower concentration of cryoprotectant. Vitrification has also proven an indispensable technique in developing advanced technologies pre-implantations genetic diagnosis/screening (PGD/PGS). Due to high survival rates and low rates of cooling injury, cryopreservation by vitrification has greatly improved the survival rate of biopsied embryos and giving a greater flexibility to PGD cycles (32).

1.2.3 Pre-implantation genetic diagnosis and screening

Pre-implantation genetic diagnosis (PGD) was first described in the medical literature in 1990 (33, 34). Since its introduction twenty years ago, PGD has grown steadily with new applications and methodology introduced regularly, becoming an established and accessible alternative to invasive prenatal diagnostic tests for many couples with specific inherited disorders. These may be single gene disorders or chromosome rearrangements. Single gene disorders may be inherited in an autosomal dominant pattern (Huntington's disease, myotonic dystrophy type 1, neurofibromatosis), autosomal recessive (Cystic fibrosis (CF), spinal muscular atrophy) or X-linked pattern (haemophilia, Fragile X) (35). In Ireland, the first live birth following PGD was in 2014. PGD was performed due to the male partner, affected by CF, being homozygous for Δf508 mutation and the female partner being

a known CF carrier (G551D mutation) giving the couple a 50% chance of a child affected by CF (36).

Pre-implantation genetic screening (PGS) is a more recent development, utilising array - comparative-genomic hybridisation (array-CGH), or more recently, next generation sequencing (NGS) in order to assess the chromosomal complement of the embryo for aneuploidy. The initial drive for PGS was to improve general IVF success rates. This was due to situations where morphologically good quality embryos were implanted but did not result in live births. The theory evolved that in spite of top quality grading based on morphology and development an embryo may be aneuploid and therefore less likely to implant and/or more likely to result in miscarriage (37). PGS has found other potential applications in cases of recurrent implantation failure, recurrent pregnancy loss and IVF in cases of advanced maternal age (associated with a higher risk of aneuploidy). PGS has been shown not to increase the live birth rate but to decrease the number of embryo transfers (by avoiding transfer for aneuploid embryos) and therefore, decrease the time to a successful live birth. The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium 2010 reported that 5,780 oocyte retrievals had been performed in 62 European centres, 2,753 for PGD and 2,979 for PGS, resulting in 997 live births (38).

1.3 How successful is ART?

Advances in the treatment process over the past 20 years have led to a steady increase in pregnancy and live birth rates. The Human Fertilisation and Embryology Authority (HFEA) in UK reported an increase in live birth rate from 14% in 1991 to 25% in 2011 (39). In Ireland, Naasan et al reported on ten years of ART data from Irish fertility clinics, demonstrating an increase in clinical pregnancy rates per cycle from 22.9% per oocyte retrieval in 1999 to 29.8% per oocyte retrieval in 2008 (see Table 1.1 and Table 1.2) (40). During that time period there was a substantial increase in the number of fertility clinics in the country, correlating with an almost three- fold increase in the number of IVF cycles. More recent data from ART in Europe ESHRE reports shows a drop in the number of fertility clinics choosing to report to ESHRE. However, there is an increase in the livebirth rate after IVF/ICSI treatment here, likely corresponding to better laboratory techniques. There is an increase in the livebirth rate from frozen embryo transfer (FET) which likely corresponds to the introduction of vitrification.

Table 1.1: Ten years of national Irish ART data

Year	No. of clinics in Ireland	No. of clinics reporting	IVF[1]	ICSI[2]	FET[3]	OD[4]	All cycles
1999	5	3	689	472	177	0	1338
2000	5	3	782	527	261	0	1570
2001	5	3	917	569	238	0	1724
2002	5	5	952	567	390	3	1912
2003	5	4	1078	618	359	3	2058
2004	5	6	1267	847	466	0	2580
2005	7	6	1429	901	524	6	2860
2006	7	6	1588	1004	636	4	3232
2007	7	6	1768	1096	692	9	3556
2008	7	5	1772	1094	682	9	3548
2009	7	6	1987	1328	744	6	4065
2010	7	6	1856	1320	882	20	4078
2011	7	5	1200	1080	762	0	3042
2012	7	4	1119	1008	706	0	2843
2013	7	3	678	517	371	0	1566
2014	**7**	**3**	**623**	**505**	**385**	**0**	**1513**

Data extracted from Naasan et al. Assisted reproductive technology treatment outcomes. Irish medical journal. 2012 May;105 (5):136 - 9 (40) and from ART in Europe, 2009, 2010, 2011, 2012, 2013 and 2014: Results generated from European Registers by ESHRE

1- IVF- in vitro fertilisation

2- ICSI- intracytoplasmic sperm injection

3- FET- frozen embryo transfer

4- OD- oocyte donation

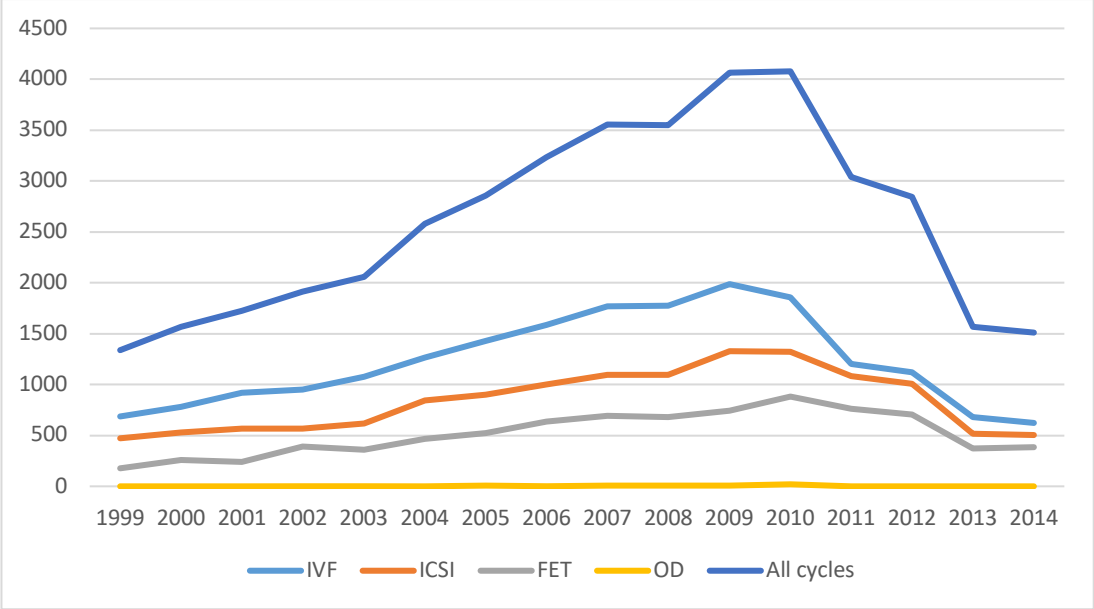

Figure 1.1: Ten years of Irish ART data in a graph.

Data extracted from Naasan et al. Assisted reproductive technology treatment outcomes. Irish medical journal. 2012 May;105 (5):136 - 9 (40) and from ART in Europe, 2009, 2010, 2011, 2012, 2013 and 2014: Results generated from European Registers by ESHRE

Table 1.2: Deliveries following IVF, ICSI and Frozen embryo transfer (FET) in Ireland 2009 – 2014.

Year	Number of clinics reporting	IVF No. of egg collections	IVF Deliveries per EC[1] (%)	ICSI No. of egg collections	ICSI Deliveries per EC[1] (%)	FET No. of embryo thawing's	FET Deliveries per thawing (%)
2009	6	1599	22.6	1137	23.1	744	15.3
2010	6	1483	25.6	1173	26.1	882	13.9
2011	5	1035	24.7	1003	21.5	762	16.3
2012	4	904	24.7	922	24.4	716	14.1
2013	3	519	33.9	498	26.5	371	20.8
2014	3	507	34.9	460	33.5	385	20.8

1 EC = egg collection

Data extracted from ART in Europe, 2009, 2010, 2011, 2012, 2013 and 2014: Results generated from European Registers by ESHRE

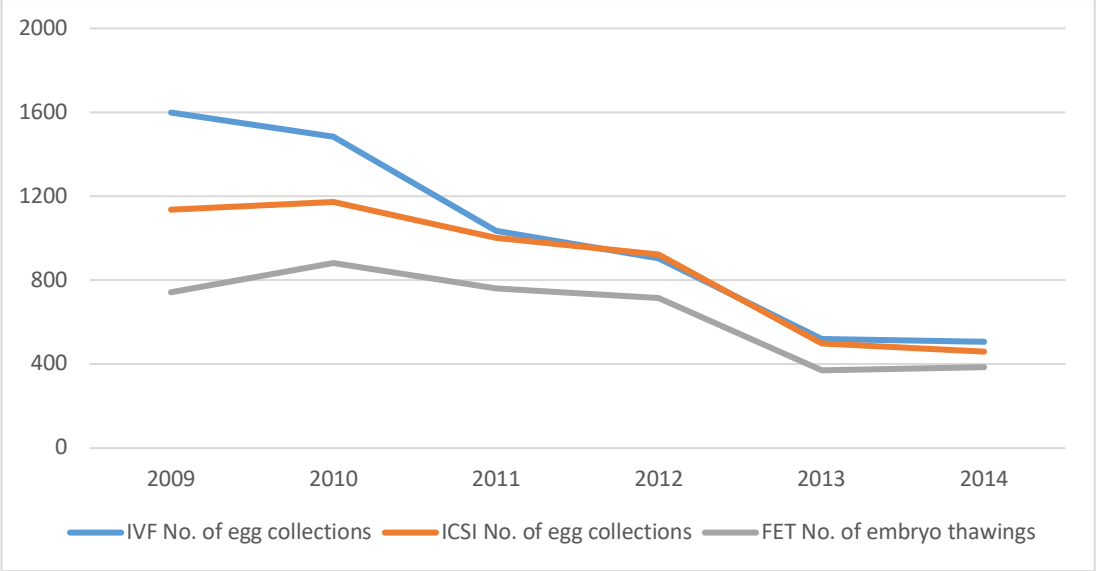

Figure 1.2: Number of egg collections (EC) for IVF or ICSI and number of embryo thawing's for Frozen embryo transfer (FET) in Ireland 2009 - 2014.

Data extracted from ART in Europe, 2009, 2010, 2011, 2012, 2013 and 2014: Results generated from European Registers by ESHRE.

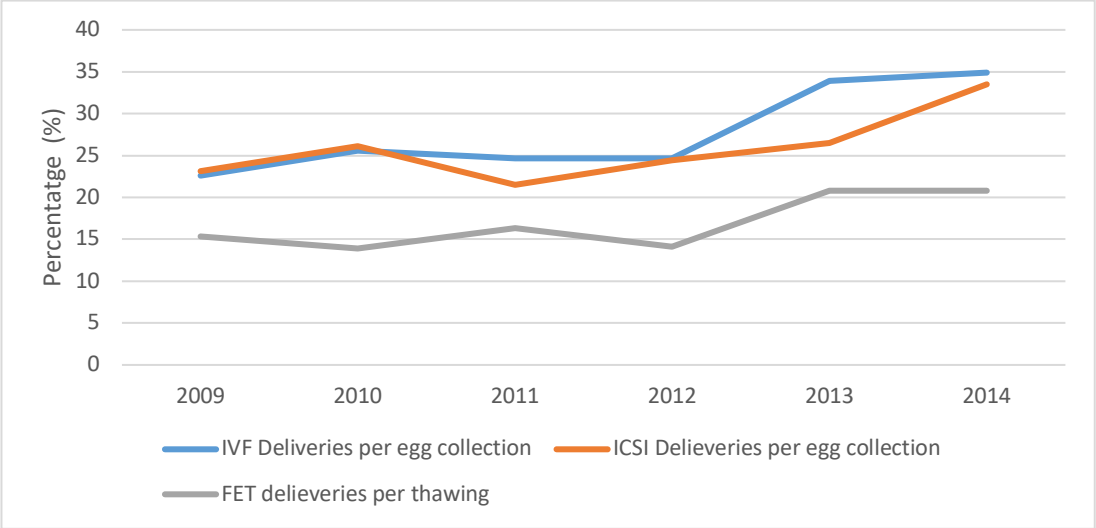

Figure 1.3: Deliveries per egg collection following IVF or ICSI and per Frozen embryo transfer (FET) in Ireland 2009 - 2014.

Data extracted from ART in Europe, 2009, 2010, 2011, 2012, 2013 and 2014: Results generated from European Registers by ESHRE.

1.4 Factors influencing successful outcome of ART

1.4.1 Age

The age of the female partner is one of the more significant determinants of ART success. In females, during fetal development, a finite number of germ-line oogonia are formed, the number of oocytes is at a maximum at 20 weeks gestation (6 - 7 million), decreasing to 1 - 2 million at birth. They remain as primordial follicles until completion of the process of meiosis, just prior to ovulation. Importantly, no new oocytes are formed during a woman's reproductive life. Conversely, the primordial follicles are recruited daily from this pool until the age of menopause, resulting in the continuous attrition of the pool of follicles (41). The rate of attrition increases with age (diminishing ovarian reserve), doubling after approximately 37.5 years, thus accelerating the decline in fertility and fecundity seen with advancing age (42). Furthermore, the quality of the remaining oocytes also diminishes with advancing age.

Fertility declines precipitously after 37 years of age, however the slow decline starts much earlier, with recent studies suggesting that women aged 19 - 26 years have twice the chance of spontaneous pregnancy compared with women aged 35 - 39 years (43). Female age is one of the most significant determinants of ART success. IVF requires the retrieval of a number of mature oocytes to result in the development of an embryo of sufficient quality to achieve live birth. Therefore, the decline in oocyte numbers and quality seen in older women results in a corresponding decline in ART success in these women. The HFEA report of Fertility Treatment Trends and

Figures 2012 illustrates this age-related decline in live birth rate from IVF /ICSI treatment (see Table 1.3) (39).

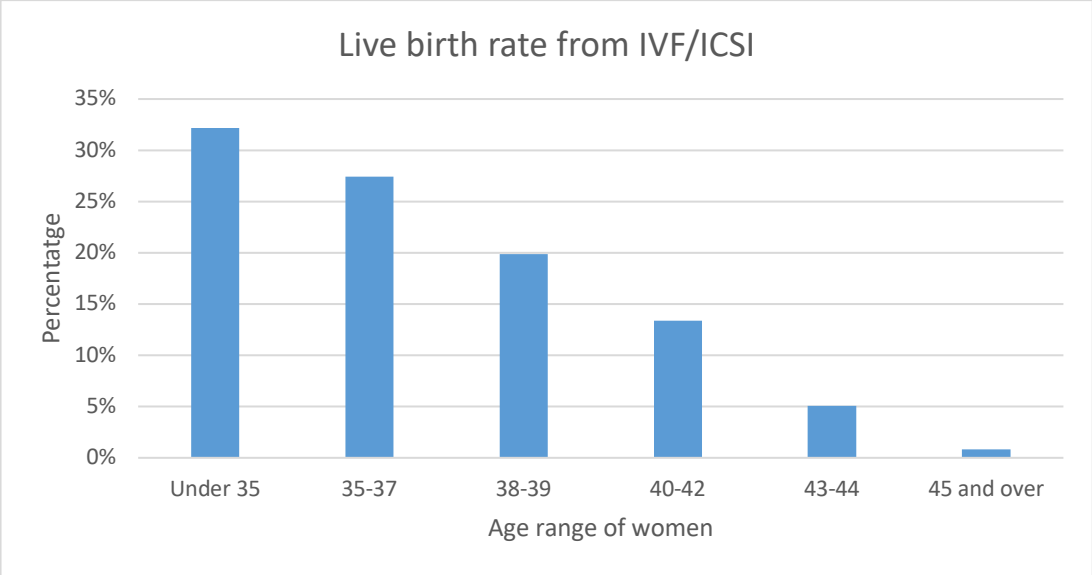

Figure 1.4: Live birth rate (%) from IVF/ICSI treatment according to age range of women.

Table 1.3: Live birth rate (%) from IVF/ICSI treatment according to age range of women.

Age range of women	Live birth rate from IVF/ICSI
Under 35	32.2%
35 - 37	27.4%
38 - 39	19.9%
40 - 42	13.4%
43 - 44	5.1%
45 and over	0.8%

Data from Fertility Treatment Trends and Figures 2012, HFEA. (39)

1.4.2 Ovarian reserve

Ovaries contain three populations of follicles: primordial (< 0.05mm in diameter), early growing (< 2mm in diameter) and antral follicles (> 2mm in diameter). It is the antral follicles that are highly sensitive to FSH. In the natural menstrual cycle, it is in the form of endogenous FSH, resulting in the development of a dominant follicle and the remainder of the antral follicles undergo atresia. In an IVF cycle it is in the form of high dose exogenous FSH resulting in stimulation of multiple follicles.

The number of oocytes available for ovulation, i.e. the antral follicles (2 - 10mm in diameter) are visible using transvaginal ultrasonography. The number of antral follicles (antral follicle count; AFC), reflects the number of remaining primordial follicles and therefore gives a good indication of ovarian reserve. AFC also gives an indication of the response to gonadotrophin stimulation and therefore an indication of oocyte yield at oocyte retrieval with subsequent impact on chance of live birth (44).

Serum FSH levels performed in the early follicular phase of the menstrual cycle (day 2 - 4) were the traditional biochemical test of ovarian reserve. High FSH levels (> 10 IU/L) may indicate a reduced ovarian reserve, with FSH > 15IU/L suggesting that the chance of ovulating a fertile oocyte are extremely low and FSH > 25IU/L indicating menopause or premature ovarian insufficiency. Higher levels of FSH also indicate poor response to exogenous FSH stimulation and therefore a poor oocyte yield at oocyte retrieval. However, due to marked inter-cycle variability in FSH levels, a single measurement is not reliable in predicting ovarian reserve or response

to stimulation, particularly in women under 40 years of age. Indeed a woman with diminished ovarian reserve may not demonstrate an elevated FSH level (45).

Anti-Müllerian hormone (AMH) level has superseded FSH as a biochemical marker of ovarian reserve. AMH is produced by the pre-antral and antral follicles of the ovary and for that reason is indicative of a woman's ovarian reserve. It does not display the inter- and intra- cycle variability of FSH and therefore a single measurement is more useful (Table 1.4).

Table 1.4: Anti-Müllerian hormone level ranges and corresponding fertility potential.

Fertility	**AMH level (pool/L)**
Very low/ undetectable	< 3.07
Low	3.08-21.97
Satisfactory	21.98 -40.03
Optimal	40.04 - 67.9

AMH has become a useful test in clinical practice as it has been shown to be predictive of ovarian response to stimulation. This is useful in predicting chances of successful outcome of IVF treatment in terms of oocyte yield but also in determining dose of gonadotrophin stimulation. In cases of very high AMH, often women with polycystic ovaries, it indicates a reduced dose of stimulation is required to avoid the risk of ovarian hyperstimulation syndrome (OHSS), whereas a low AMH indicates diminished ovarian reserve (DOR) and a requirement for an increased dose of gonadotrophins (46). Ovarian reserve declines with ovarian ageing, however this decline may not always correlate with increasing female age, i.e. a decline in ovarian reserve that is out of keeping with chronological age can be seen in younger women.

There is wide consensus in the medical literature regarding the effect of maternal age on fertility. Advancing male age however, is much less studied with less clear consensus on the role of advancing paternal age and fertility.

Although it is known that men can father a child into their 7^{th} and 8^{th} decades of life, there is evidence of subtle changes in male fertility potential. Several studies have demonstrated a longer time to natural conception amongst older men, > 35 years, when compared to younger men and controlled for maternal age (43, 47-49). Semen parameters however, are not significantly affected by paternal age. A review by Dain et al showed agreement amongst four studies for a decline in semen volume and an associated decline in total sperm count and total motile sperm due to the reduced semen volume but no significant relationship between advancing age and sperm concentration, motility or morphology (50). However, several other studies have found an effect of paternal age on sperm motility (51-53).

Maternal age significantly affects IVF/ICSI outcomes and for this reason it is difficult to clearly elucidate the possible effects of paternal age on ART outcomes. Several studies indicate an effect, when adjusted for maternal age, demonstrating a decline in live birth rates with advancing male age (54-56). Interestingly, some studies reported on the combined effect of advanced maternal and paternal age resulting in lower pregnancy rates and raised the question regarding the ability of an older oocyte to repair DNA damage in older spermatozoa (55). To this end, numerous groups have reported on the impact of paternal age in cycles using donor oocytes, as donor oocytes are generally from younger women. The results are mixed, further indicating the complex role of the female factor on ART outcome (52, 57-60).

1.4.3 Body Mass Index

Obesity has been identified as an important factor in general health. For women of reproductive age, it has important implications for pregnancy health, associated with gestational diabetes and intrapartum morbidity. In Ireland, the rates of obesity continue to rise, with almost one in five women now obese according to a study from a large tertiary maternity hospital (61).

The most commonly used measurement of weight is body mass index (BMI) measured as kg/m^2 (see Table 1.5).

Table 1.5: Body Mass Index (BMI) ranges.

BMI category	**BMI Range (kg/m^2)**
Underweight	< 18.5
Normal	18.5 - 24.9
Overweight	25 - 29.9
Obese Category I	30 - 34.9
Obese category II	35 - 39.9
Obese category III	≥ 40.0

Women who are overweight or obese have lower spontaneous pregnancy rates compared with their normal weight counterparts (62). Much of this discrepancy is linked to higher rates of polycystic ovarian syndrome amongst overweight and obese women with over one-third of with polycystic ovarian syndrome (PCOS) obese, leading to alterations in the hypothalamic-pituitary axis and affecting

frequency and regularity of ovulation (63). There exists significant debate however about the effects of overweight and obesity on success rates from IVF treatment. A systematic review of studies of overweight and obesity effect on IVF suggest the requirement for higher doses and longer duration of gonadotrophin stimulation, lower clinical pregnancy rates, higher miscarriage rates and reduced live birth rates amongst this population when compared to women of normal BMI (64).

Obesity has been shown to be a risk factor for poor obstetric outcomes, both maternal and fetal. These include gestational diabetes, gestational hypertensive disorders, increased rates of caesarean section, miscarriage, intrauterine fetal demise, fetal macrosomia and low birth weight. One study reported discrepancies in obstetric outcome according to mode of conception at normal and overweight BMI, with IVF-conceived pregnancies more likely to be complicated by placental ischaemic disorders and low birth weight. Among the women in the obese category however, the risks of adverse outcome were not significantly different according to mode of conception, indicating that obese category BMI has a significant impact on pregnancy outcome irrespective of mode of conception (65).

Bodyweight plays an important role in the initiation of menarche and the maintenance of regular menstrual cycles. In order for puberty to progress normally a BMI of > 19 kg/m^2 must be achieved and to maintain regular cycles a woman's body should be comprised of at least 22% fat. Underweight also poses a risk to pregnancy with increased risk of small for gestational age infants (66).

1.4.4 Smoking

Cigarette smoking has a detrimental effect on health and fertility. The metabolites of cigarette smoke cause oxidative damage to oocytes, sperm and embryos. Several studies have identified cigarette smoking as having negative effects on infertility and time to conception. A meta-analysis included 12 studies totalling 10,928 female smokers and 19,179 female non-smokers found that smokers were significantly more likely to be infertile (OR: 1.60, 95% CI: 1.34 – 1.91) (67).

Menopause occurs one to four years earlier in smoking women than in non-smokers, likely secondary to acceleration of ovarian follicular depletion. Mean FSH levels are significantly higher in young smokers than in non- smokers. Luteal phase urinary oestrogen in smokers is only about one-third that observed in non- smokers. Current smoking is also associated with lower AMH levels in late reproductive age and perimenopausal women (68).

Mean gonadotropin dose requirements for smokers receiving stimulation for in vitro fertilisation (IVF) are higher when compared with those of non-smoking women.

1.4.5 Nutrition

A varied diet is important for maintaining general health and a healthy BMI. A balanced diet supplies the nutrients necessary for activation of enzymes involved in DNA synthesis. Several of these enzymes are zinc- and vitamin B- dependent. Furthermore, a diet deficient in B vitamins- folate, B6 and B12- results in an increased

concentration of homocysteine. Hyperhomocysteinaemia has been shown to be detrimental to IVF/ICSI cycle success. Elevated levels in follicular fluid, aspirated at the time of oocyte retrieval, are negatively associated with oocyte numbers and embryo quality (69, 70).

Diets with high intake of fruit, vegetables, fish and vegetable oils are higher in folate, vitamin B6 and certain essential fatty acids e.g. linoleic acid (a pre-cursor for certain prostaglandins necessary for pre-antral follicle development and ovulation). Such diets are associated with improved IVF outcomes (71, 72). Several studies also show a positive effect on sperm parameters, specifically progressive motility, in diets higher in fruit, vegetables and cereals (73-76).

This Mediterranean style diet is also a source of antioxidants- vitamin E, vitamin C, beta-carotene, folate and B vitamins- which reduce the amount of damaging reactive oxygen species (ROS). Micronutrients such as zinc, selenium, manganese, magnesium and copper also play a role, as co-factors of enzymes, in the antioxidant effect on ROS (77). ROS are damaging to gametes and spermatozoa, in particular, are dependent on the antioxidants and DNA repair enzymes in seminal plasma for protection against ROS and the resultant sperm DNA fragmentation and membrane damage (78). It is by mitigating against this damage that antioxidants are proposed to play a role in improving fertility. There remains no consensus however on the impact of sperm DNA fragmentation on IVF outcomes (79, 80).

Therefore, it is not surprising that there have been several studies into the benefits of antioxidant supplementation in males and females with mixed results. A Cochrane review of antioxidant supplementation in females found that the low to

very low-quality evidence demonstrated no improvement in live birth rate or clinical pregnancy rate. In three studies, pentoxifylline was the only antioxidant that demonstrated some evidence of a higher clinical pregnancy rate (81). Antioxidant supplementation in males was also the subject of a Cochrane Review. Four randomised controlled trials (RCT) demonstrated a benefit in terms of live birth but the numbers in the studies were small and the quality of the evidence was deemed low. Similarly, seven low quality RCTs with small numbers suggested an increase in clinical pregnancy rate associated with male antioxidant supplementation (82).

Supplementation with folic acid for women of 400mcg daily in the preconception period and up to 12 weeks gestation has demonstrated a reduction in neural tube defects in the fetus (83, 84). In men with severe oligozoospermia, there is some evidence for folic acid and zinc in combination in improving sperm concentration, although there is no consensus on the optimum dosage (85).

Vitamin D is the 'wonder nutrient' of our time and vitamin D deficiency has been implicated in a variety of medical conditions. Dietary vitamin D is found in such food sources as oily fish, egg yolks and fortified products (milk, cheese, orange juice). The other major source of vitamin D is sun exposure. Due to modern diet and indoor, sedentary lifestyle, there is an increasing prevalence of vitamin D insufficiency and deficiency. Of particular concern is the re-emergence of rickets due to the increasing prevalence of deficiency in pregnancy, particularly in ethnic minority groups.

There have been several studies on the role of vitamin D in fertility, with two recent systematic reviews of the evidence (86, 87). Vitamin D is a steroid hormone, the effects of which are mediated through the vitamin D receptor (VDR). These

receptors are found throughout the body. In females there are receptors in the ovaries and endometrium and in males in the epididymis, seminal vesicle, prostate and in the nucleus of spermatozoa.

In the female, vitamin D has been shown to stimulate steroidogenesis, in one study of the effects of the active metabolite of vitamin D, 1,25 $(OH)_2D_3$, on human ovarian tissue, found it increased progesterone production by 13%, oestrone by 21% and oestradiol by 9% (88). It also plays a role in endometrial receptivity and in the placenta with 1,25 $(OH)_2D_3$ involved in the regulation of placental lactogen, HCG expression and secretion in human syncytiotrophoblasts, calcium transport in the placenta, as well as decidualisation of the endometrium (89-91).

Several studies have found a positive correlation between vitamin D levels and AMH levels. In particular, that vitamin D supplementation prevents the seasonal variation that AMH levels have displayed in various studies (92-94).

Polycystic ovarian syndrome (PCOS) has been much studied in the context of vitamin D. PCOS is characterised by oligomenorrhoea, morphologically polycystic ovaries on ultrasound, increased ovarian and adrenal androgen secretion resulting in hyperandrogenic symptoms (hirsutism, acne). Many women with PCOS will be overweight or obese and demonstrate insulin resistance. Several studies have demonstrated that vitamin D deficiency is more common amongst women with polycystic ovarian syndrome when compared with healthy controls (95, 96). However, it is difficult to clearly elucidate how significant the contribution vitamin D makes to the PCOS phenotype, particularly obesity and insulin resistance (97). These studies raise the suggestion that vitamin D measurements and supplementation may

have a role in the standard management of PCOS and associated ovulatory dysfunction.

In males the role of vitamin D is less clear. Calcium plays an important role in spermatogenesis, sperm motility and the acrosome reaction of fertilisation. Blomberg at al demonstrated an association between serum vitamin D levels and increased intracellular Ca^{+2} concentration and sperm motility as well as inducing the acrosome reaction (98). Other authors have not found such an association but suggested that low and high serum levels of vitamin D had a negative effect on sperm count, sperm motility and morphology (99).

Vitamin D and IVF outcome has been investigated by several authors, without reaching a clear consensus. Ozkan et al. found that women with higher serum and follicular fluid 25 (OH) D levels had higher implantation rates and a significantly higher clinical pregnancy rate following IVF. The study also found that each 1ng/mL increase in follicular fluid concentration of 25 (OH)D increased by 6% the chance of clinical pregnancy (100). Several other studies however have found no clear association between serum or follicular levels of 25 (OH)D and IVF outcomes (101, 102).

Interestingly, Budick et al reported on the impact of serum vitamin D levels in donor oocyte cycles. This study found that the vitamin D deplete recipients had a significantly lower rate of clinical pregnancy and live birth (37% v 78% and 31% v 59% respectively) when compared to vitamin D replete recipients. These findings suggested vitamin D may play a more important role through the effects it has on the endometrium than those on the follicle or oocyte (103). A similar study in the same

year however showed no difference in pregnancy rates or ongoing pregnancy rates between vitamin D replete and deplete oocyte recipients (104).

There is certainly a suggestion that vitamin D plays a role in the reproductive process in males and females but due to the lack of clarity on the exact mechanism by which it exerts its effect there remains no consensus on the benefit of vitamin D measurements and supplementation in the IVF population.

1.4.6 Alcohol

Alcohol consumption is associated with adverse health effects, however, the effect of alcohol on fertility is difficult to establish, particularly the level of alcohol consumption at which it becomes harmful. Most studies in the literature indicate a detrimental effect on fertility. A prospective study by Jensen et al reported-on fecundity of 430 Danish women, over six menstrual cycles where alcohol consumption was monitored over 1 week each month. The study found that the chance of a successful conception decreased with increasing alcohol intake, OR 0.61 (10 - 50g alcohol per week) to 0.34 (100g alcohol per week) (105). Similar findings were reported by Hakim et al in another prospective study of 124 ovulating women, this time combined with cigarette smoking. Non-drinkers of alcohol who did not smoke had a rate of conception of 24.5%, compared with 21.6% for non-drinkers who smoked, 14.3% for women drinking 1 - 12g alcohol per week, 10.5% (13 - 90g alcohol per week) and 10.9% (> 91g alcohol per week).

A Danish study by Juhl et al of 29,844 planned pregnancies, where 79% of women reported up to 7 drinks per week (where one drink equalled 12g alcohol) and 12% reported no alcohol intake at all in the time leading up to pregnancy, found no significant correlation between moderate alcohol consumption and increased time to pregnancy. In contrast to other studies, they identified a possible detrimental effect of no alcohol consumption on time to pregnancy.

High levels of alcohol consumption in males is associated with profound effects on Leydig cell function by decreasing testosterone synthesis and membrane damage resulting in the development of Leydig cell auto-antibodies due to the presence of acetaldehyde. However, reports in the medical literature also demonstrate conflicting results at moderate alcohol intake levels with some studies indicating no effect on sperm parameters (106-108) and other studies indicating harmful effects (109-111).

There are several studies in the literature demonstrating a detrimental effect of alcohol consumption, by both the male and female partner, on the success rates of IVF treatment. One study found that female alcohol consumption was associated with lower oocyte numbers at retrieval (OR 0.87, CI 0.77 - 0.98, p = 0.02), chance of pregnancy (OR 2.86, CI 0.99 - 8.24, p = 0.05) and increased risk of miscarriage (OR 2.2, CI 1.09 - 4.49, p = 0.03). For men, one additional alcoholic drink per day in the month leading up to oocyte retrieval increased the risk of not achieving a live birth 2.28 fold (1.08 - 4.80) and to 8.32 fold (1.82 - 37.97) in the week of oocyte retrieval (112).

1.4.7 Exercise

Exercise, in moderation, is an essential part of a healthy lifestyle and has no negative impact on reproductive health (113, 114). Managing weight gain through moderate exercise is beneficial, not only to reproductive health, but to general medical health.

In terms of achieving a pregnancy, moderate exercise increases insulin sensitivity thus reducing carbohydrate-induced hyperinsulinaemia, which may favour embryo implantation. Vigorous exercise however, reduces leptin, which may play an important role in regulating embryo implantation (115).

There is much research showing that exercise at extreme levels, such as that undertaken by female athletes, does have an impact on fertility. Vigorous exercise can cause a hypothalamic dysfunction leading to effects on gonadotrophin- releasing hormone (GnRH) pulsatility. The reduction in GnRH pulsatility results in a limited LH and, to a lesser extent, FSH release from the pituitary gland. The result is lower ovarian follicle stimulation, lower levels of oestrogen release, anovulation, oligomenorrhoea and luteal phase defects thus effecting chances of conception (116, 117).

Several studies have reported on physical activity amongst non-athletes and its effect on reproductive function and fecundity. Three studies have evidence of lower leptin and progesterone concentrations, particularly in the luteal phase, amongst women with high levels of physical activity (118-120). Similarly, in some studies of fecundability, high levels of physical activity have been shown to have

negative effects on the chance on conception, while moderate exercise has a beneficial effect (121, 122).

For those undergoing ART, specifically IVF/ICSI, the findings are somewhat similar, though again, are mixed. High levels of regular exercise, 4 hours per week for less than 10 years, were shown, in one study, to be associated with lower levels of live birth when compared to women not engaged in regular physical activity (123). Gaskins et al however, found regular moderate-to- vigorous levels of exercise (2.5 hours per week) to have no negative effect on success from IVF and that certain aerobic exercises had a positive effect (124). Moderate exercise levels before or during an IVF cycle show positive effects on pregnancy/live birth, with benefit shown particularly in overweight/obese women (121, 125, 126).

1.4.8 Emotional wellbeing and social support

Psychological stress is prevalent amongst couples trying to conceive and undergoing assisted fertility treatment. Not only does the process of trying to achieve a pregnancy spontaneously become stressful with increasing time to conception but the weight of expectation associated with fertility treatment is a source of great stress.

It has long been recognised that physical (excessive exercise) and psychological stress in a woman of reproductive age manifests physically in disrupted menstrual cycles due to the effect on the hypothalamic-pituitary axis (127). Stress hormones such as catecholamines (adrenalin, noradrenaline and dopamine) and the

hypothalamic-pituitary-adrenal axis interact with hormones which are responsible for normal ovulatory cycles: i.e., gonadotropin releasing hormone (GnRH), prolactin, LH and FSH. Hypothalamic-pituitary axis activation leads to increased cortisol production and a delay or inhibition of pre-ovulatory GnRH and LH surge. This results in delayed ovulation, a shortened luteal phase and irregular menses, limiting the opportunity of the female to conceive (128, 129).

There is no doubt that couples undergoing IVF treatment feel stress and that it is one reason for dropout rates from treatment (130, 131). The difficulty arises when separating cause and effect, particularly when determining the effect of stress on success of IVF treatment. Reflecting this is a growing number of studies with conflicting results regarding the impact of stress on IVF success (132-135). A meta-analysis by Matthiesen et al reviewed 31 prospective studies of 4902 women, for stress or distress, including anxiety and depression. They found a small but significant effect of stress and anxiety on clinical pregnancy rates but this significance did not occur for positive pregnancy test and live birth rate. The latter might be explained by under-powered studies and the heterogeneity between studies as the authors admit that the discrepancy is not biologically plausible (136). Boivin et al followed up with a meta-analysis including 14 studies of 3583 women prior to their first IVF cycle and found no significant impact of emotional distress on the outcome of an IVF cycle. There was a significant pooled standardised mean difference indicating more distress amongst the not pregnant group when positive pregnancy test was used as the definition of pregnancy, this difference was not seen when positive ultrasound scan

or live birth were used (137). The findings of these meta-analyses reflect the difficulty in elucidating the true effect of stress on the outcome of IVF.

There is more robust data however on the effect of stress on early pregnancy success, which ultimately impacts on the success, if it is to be measured in terms of live birth, of an IVF treatment cycle. There is evidence to suggest the high perceived stress by pregnant women in early pregnancy is associated with increased rates of miscarriage. Immunological imbalances have been linked to miscarriage in those who reported high perceived stress and women who reported feeling stressed, anxious, depressed, out of control or overwhelmed in their first trimester had higher odds of miscarriage (138, 139).

1.4.9 Physical characteristics of the uterus

The exact contribution of the physical characteristics of the uterus to the outcome of ART is the relative unknown of the treatment cycle.

Uterine fibroids are one of the more accessible characteristics to study. Uterine fibroids are common, benign tumours of the uterus increasing in prevalence with increasing age and more common in certain ethnicities. Fibroids are classified according to their position in the uterus - subserosal fibroids, intramural fibroids and submucosal fibroids. Fibroids are estimated to be present in 5 - 10% of subfertile women. In 1 - 2% of subfertile couples the cause may be due to fibroids alone. In this case it is usually due to distortion of the endometrial cavity by submucosal fibroids, typically with a significant endometrial component.

A systematic review by Pritts et al on the impact of fibroids on fertility and the benefit of myomectomy indicated that subfertile women with submucosal fibroids had decreased implantation rates and clinical pregnancy rates compared with subfertile women without fibroids. It was concluded that excision of submucosal fibroids is likely to incur benefit in improving fertility outcomes. The data regarding intramural fibroids was less conclusive due to poor study quality. There is a suggestion of decreased fertility and increased rates of miscarriage amongst this group with no significant benefit from myomectomy. Subfertile women with subserosal fibroids had similar outcomes to those without fibroids (140).

Congenital abnormalities of the uterus can also affect fertility by reducing implantation and increasing pregnancy loss. Müllerian duct anomalies arise during embryogenesis, from a failure of the Müllerian ducts to either properly fuse or failure in their correct development. This results in an array of structural aberrations of the uterus. The most common abnormality is the septate uterus. This may be a mild septum bulging into the uterine cavity or the septum may extend varying distances into the uterine cavity with the most severe being a complete septum. Diagnosis is typically made by a combination of transvaginal ultrasound and laparoscopy and hysteroscopy. A septate uterus is associated with the highest incidence of subfertility and may be associated with first- and second-trimester pregnancy loss and preterm labour (141). A review by Homer at al found that in women with septate uteri, 79% of pregnancies resulted in miscarriage with the underlying mechanism being a poor blood supply to the septum thus impairing implantation (142).

In women without uterine abnormalities, little is understood about the effect of uterine factors on fertility. Uterine dimensions have been studied by few groups in order to determine the effect of uterine length on pregnancy outcome following IVF. Many of the studies were performed in order to determine the optimum distance into the uterine cavity at which to place embryos during embryo transfer. The length of the uterus was determined by uterine sounding using a uterine sound or the embryo transfer catheter. The length was therefore from the external os to the uterine fundus. Egbase et al reported highest implantation and clinical pregnancy rates in women with a uterine length of 7 - 9cm, although the findings were not significant. The study also reported a non-significant finding of a higher rate of ectopic pregnancy among the group with smaller (< 6cm) uteri (143).

Hawkins et al studied the uterine length of 5120 women by uterine sound in the cycle preceding IVF treatment and the subsequent pregnancy outcomes according to length. Of note, the study did not record parity of the study participants. The median length of the uterus was 7cm. Women with small uteri (< 6cm), when compared with those in the referent group (7 - 7.99cm), were half as likely to achieve a live birth (RR: 0.53, 95% CI: 0.35 - 0.81) and more likely to have spontaneous miscarriage (RR 2.16, 95% CI: 1.23 - 3.78). Those with uteri 6 - 6.99cm, when compared to the referent group were also less likely to achieve a live birth (RR: 0.91, 95% CI: 0.85 - 0.98). The study also found that women with larger uteri > 9cm had a non-significant reduction in chance of live birth with compared to the referent group. There was a positive association with higher BMI > 30kg/m^2, increasing quartiles of weight and increasing quartiles of height and larger uterine lengths. The authors

suggesting that larger uteri act as an intermediate in the relationship between obesity and decreased success from fertility treatment (144).

Ultrasound is routinely used in IVF/ICSI cycles to assess the endometrium and to monitor follicular growth in order to time oocyte retrieval. There has been much focus on three-dimensional evaluation of the endometrium and endometrial vascularity in order to predict pregnancy from IVF treatment (145-147). There are no published data on pre-conceptual ultrasound evaluation of the uterus as an aid to predict preterm delivery, particularly in twin pregnancy. One group reported ultrasound evaluation of the uterus (hysterosonometry) prior to conception to predict preterm birth by measuring the size of the uterus (external so to fundus). The study was small (n = 79) however, they reported a statistically significant correlation between smaller uteri (< 63mm in length from external cervical os to fundus) and preterm delivery (148). The advent of three-dimensional ultrasound has allowed more accurate assessment of uterine volume when compared to two-dimensional ultrasound (149).

1.5 Risks of ART

ART due to the invasive nature of the treatment carries small risks of sepsis secondary to oocyte retrieval and trauma to intra-abdominal organs at the time of oocyte retrieval (150, 151).

1.5.1 Ovarian hyperstimulation syndrome

The most significant risk of ART is the risk of ovarian hyperstimulation syndrome (OHSS) associated with superovulation with gonadotrophins. Women most at risk are those with polycystic ovaries and women under 30 years of age i.e. those with a good ovarian reserve (high AFC or high AMH). The true incidence is difficult to establish as there is no requirement for mandatory reporting of mild – moderate OHSS but is estimated to occur in 1 - 10% of IVF patients, with severe cases estimated at 0.25 - 2%. OHSS can lead to significant morbidity and is potentially fatal, particularly if poorly managed (152).

The pathophysiology of OHSS is not fully understood. It involves a sudden increase of vascular permeability in response to hCG or LH following super-ovulation with gonadotrophins. The vascular permeability results in third-spacing of fluid and intravascular depletion and haemoconcentration. Haemoconcentration results in an increased risk of thromboembolism. The third spacing of fluids most frequently clinically manifests as ascites and less commonly pleural or pericardial effusions.

OHSS is graded according to severity- mild, moderate, severe and critical- according to the severity of symptoms, ovarian size, degree of ascites and haematological changes.

There are two subtypes of OHSS- early and late- according to the time of occurrence in relation to the number of days from oocyte retrieval. Late OHSS always occurs in the presence of hCG from a pregnancy and therefore the condition may be more protracted. Early OHSS in the absence of a pregnancy occurring (no embryo

transferred in anticipation of OHSS or unsuccessful embryo transfer) is more likely to be self-limiting (153, 154).

1.5.2 Multiple pregnancy

Multiple pregnancy comprises twin pregnancy and higher order multiple (HOM) pregnancy including triplets, quadruplets etc. All multiple pregnancies are described according to their zygosity. A dizygotic twin pair results from two separate fertilised ova whereas monozygotic twins result from a single fertilised ovum. The twin pair is further described according to the chorionicity. All dizygotic twins have two placental masses, which are usually separate, and separate amniotic sacs and are described as dichorionic diamniotic twins. The chorionicity of monozygotic twins depends on the timing of embryo division- dichorionic diamniotic (3 days), monochorionic diamniotic (4 to 8 days), monochorionic monoamniotic (8 to 12 days) and conjoined twins (division after 13 days) (155). Monozygotic twin rates have remained relatively stable over time at approximately 4 per 1,000 maternities (156). Spontaneously conceived dizygotic twins are associated with increasing maternal age, higher parity, and family history of twinning, all thought to be related to higher circulating levels of FSH in the mother (157, 158). The worldwide rate of dizygotic twinning has increased substantially over the past number of decades due to an increase in iatrogenic twinning associated with ART treatments, particularly IVF. Monozygotic twinning is also increased with IVF (two to five fold) (159). The increased frequency of monozygotic twins may be associated with breaks in the zona pellucida associated with handling the embryo in the laboratory for techniques such

as ICSI or associated with other laboratory conditions such as culture medium (158). Dizygotic twinning from ART is associated with the transfer of two embryos into the endometrial cavity.

It is widely accepted that multiple pregnancy, irrespective of mode of conception, is associated with a higher rate of both maternal and perinatal morbidity and the risk of same increases with increasing number of fetuses in the pregnancy (160, 161). Perinatal morbidity and mortality is increased 4- to 10- fold in twins compared to singletons (162). The increased perinatal morbidity and mortality are largely secondary to the higher rate of pre-term delivery (< 37 weeks gestation). The preterm prediction study found that, of 142 twin pregnancies prospectively followed to delivery, 54% delivered before 37 weeks, 32% before 35 weeks and 8.8% before 32 weeks (163).

Antenatally, multiple pregnancies have a higher rate of intrauterine growth restriction, small for gestational age and congenital malformation than singletons. Maternal obstetric complications occur three to seven times more often in multiple pregnancy compared with singleton pregnancy and in higher order multiple (HOM) pregnancies compared to twin pregnancy (164). Maternal morbidity includes, pregnancy-induced hypertension (PIH), pre-eclampsia (PET), gestational diabetes, antepartum- and postpartum- haemorrhage (APH/PPH). Chorionicity is an important indicator of perinatal morbidity and mortality. Monochorionic twins are associated with higher rates of perinatal morbidity and mortality, particularly for the fetus when compared to dichorionic twins (165). Many of the monochorionic-specific complications are due to their interdependency on the shared placenta. Discordance

in blood supply between the twins can result in discordance in size and amniotic fluid volume, as in twin to twin transfusion syndrome (TTTS). Death of one twin can result in a 20% risk of death of the surviving twin. It can also result in a 20% risk of ischaemic lesions in the brain of the surviving twin (166).

Many studies have focused on the impact of mode of conception on risk of adverse obstetric or perinatal events with conflicting results. Several studies have reported an increased risk of low birth weight, preterm birth (167-171) whereas other studies reported no increased maternal nor perinatal risk conferred by mode of conception (172-175). Indeed, further highlighting the lack of consensus, in one systematic review by Helmerhorst et al. (176) perinatal mortality in twins conceived using ART was 40% lower compared with spontaneously conceived twins. Several studies have found that ART conceived twin pregnancies had comparable rates of maternal antenatal complications when compared to spontaneously conceived twin pregnancies, specifically gestational diabetes mellitus, gestational hypertensive disorders (pregnancy-induced hypertension and pre-eclampsia) and obstetric cholestasis (167-169, 175, 177).

Multiple pregnancies have a significantly higher rate of preterm delivery and the degree of prematurity dictates the likelihood of severe neonatal morbidity both short-term and long- term. The majority of triplets and all other higher-order multiples are delivered prematurely irrespective of mode of conception.

Multiple pregnancies also carry a significantly higher rate of delivery by caesarean section, with all triplets and higher order multiples requiring delivery by caesarean section. Several studies have reported a significantly higher rate of

caesarean delivery amongst ART twins compared to spontaneously conceived twins (178-181). However, some studies have found no significant difference in the rate of caesarean delivery according to mode of conception (168, 170, 175, 182). Several of these studies report a very high caesarean section rate (in the order of 90%) whereas Lambalk et al. report a much lower rate of caesarean delivery (30% and 37%), indicating that institutional differences in management are likely to have a role in the variation of results.

In twin pregnancies, data from several studies and meta-analyses indicate a higher rate of moderate preterm delivery amongst ART conceived twins (5, 183). A meta-analysis by McDonald et al., where three studies reporting on moderate preterm delivery in ART twins found a combined OR of 1.48 (95% CI 1.05 - 2.10) when compared with spontaneously conceived twins (183).

European Society for Human Reproduction and Embryology (ESHRE) collects outcome data for all IVF/ICSI pregnancies from the reporting fertility clinics. The graph below demonstrates that twin and triplet pregnancies carry a high rate of preterm (< 37 weeks) delivery whereas the singleton preterm delivery rate remains low. The overall preterm delivery rate for all infants across Europe ranges from 5.4 - 12.0% (184).

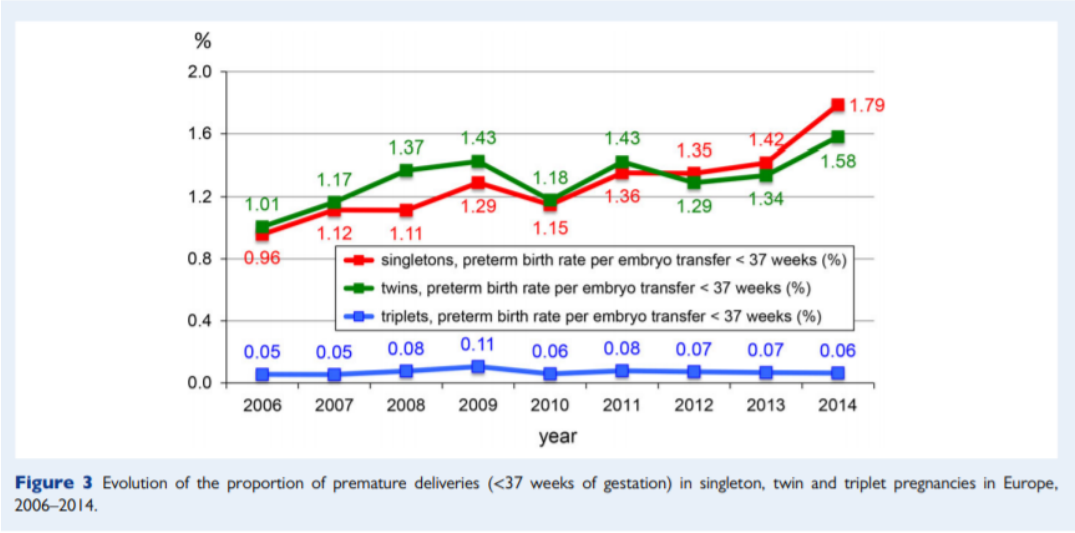

Figure 3 Evolution of the proportion of premature deliveries (<37 weeks of gestation) in singleton, twin and triplet pregnancies in Europe, 2006–2014.

Figure 1.5: Evolution of the proportion of premature deliveries (< 37 weeks gestation) in singleton, twin and triplet pregnancies arising from IVF/ICSI in Europe 2006 - 2014.

Figure extracted from ART in Europe 2014: Results generated from European registries by ESHRE. Human Reproduction, Sept 2018, Vol.33, No.9 pp. 1586–1601, 2018

The medical literature indicates that multiple pregnancy outcome is improving with more standardised care and that mode of conception does not significantly impact on the rate of complications. However, there is no doubt that multiple pregnancy carries a significant burden of risk and for this reason should remain an adverse outcome in ART.

1.6 Multiple pregnancies and ART- current practice

Since the introduction of assisted conception, in particular in vitro fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI), there has been a large increase in the number of multiple pregnancies internationally. Multiple pregnancy, specifically higher-order multiple pregnancy (HOM), arising from ART is now deemed an adverse

outcome of the treatment due to impact on both maternal and fetal morbidity and mortality (22, 185). IVF treatment involves decision-making regarding the number of embryos to transfer into the uterus. Historically, in a bid to increase pregnancy rates from treatment for couples and, as a consequence, the success rates of a clinic, it was thought best to transfer two and often three embryos into the uterus. This embryo transfer policy resulted in a surge in twins and HOM pregnancies (triplets and greater) in obstetric units. In the U.S. from 1980, the beginning of the ART era, the total number of HOM pregnancies increased over five-fold within 18 years, with similar trends seen elsewhere in the world (186, 187). Another significant change was the number of multiple pregnancies occurring in older women, who have a higher background rate of obstetric and perinatal complications, with twin birth rates in the U.S. rising 27% for women < 20 years, 80% for women in their 30s and 190% for women 40 years or older (188).

The affordability of treatment can also influence patient choices and clinical practices, specifically there is evidence from the US, where robust health insurance will cover the cost of treatment and conversely where, without health insurance, the cost to the patient is very high, that health insurance coverage will impact on the numbers of embryos transferred and subsequent multiple pregnancy rates (189). Interestingly, there is evidence to suggest that cost of a state-funded reimbursement plan for couples, even in countries where the reimbursement is substantial, are relatively modest. In a review by Chambers et al of the cost of treatment in developed countries, ART treatment accounted for 0.25% or less of total healthcare expenditure

in all countries surveyed, including countries who offered state funding (e.g. Scandinavia, Australia) and those with no public finding (United States) (190).

In these countries providing state funding there is more regulation and oversight on treatment outcomes, including stricter regulation on the numbers of embryos transferred, in order to maintain a low rate of multiple pregnancy, as the evidence shows that the cost of multiple pregnancy substantially exceeds the cost of providing ART reimbursement (191, 192).

A number of studies have estimated the healthcare costs of care provided for ART pregnancies and deliveries and have demonstrated that the economic burden for twins and HOM pregnancies is substantially greater, in the region of 3 - 4 fold, than that of a singleton pregnancy (193).

The increasing rates of multiple pregnancy as a result of ART led to a call by the relevant governing bodies for a decrease in multiple embryo transfer in order to reduce the number of twin and HOM pregnancies. Since 2009, the Human Fertilisation and Embryology Authority (HFEA) in the UK has been working to reduce the multiple birth rate as a result of ART, from 24% in 2008 to 15% in 2015, with an ultimate aim of less than 10%, which was achieved in 2017 (194, 195). Elective single embryo transfer (eSET) is the recommended policy for women less than 37 years of age or those with several good quality embryos (18). In the UK HFEA have reported that eSET rates have continued to rise (from less than 5% in 2008 to 29% in 2015) without a decline in pregnancy rates (194). Across Europe there has also been a steady rise in the number of single embryo transfers as displayed in the graph below.

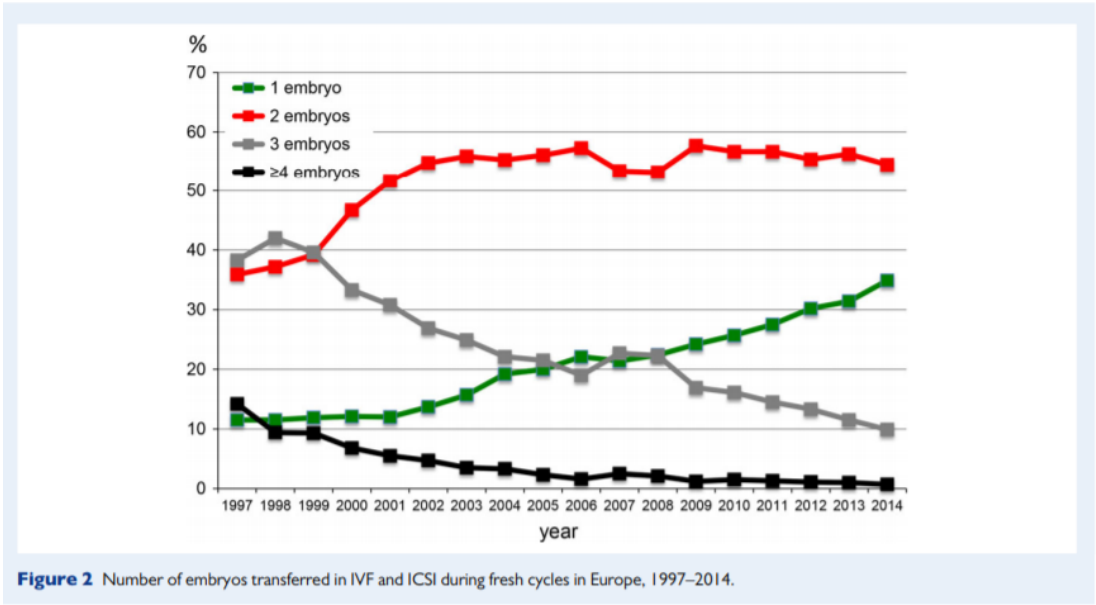

Figure 2 Number of embryos transferred in IVF and ICSI during fresh cycles in Europe, 1997–2014.

Figure 1.6: Number of embryos transferred in IVF and ICSI during fresh cycles in Europe, 1997–2014.

Figure extracted from ART in Europe 2014: Results generated from European registries by ESHRE. Human Reproduction, Sept 2018, Vol.33, No.9 pp. 1586–1601, 2018

This is also due to the improvements in cryopreservation techniques, namely vitrification. This means that one IVF cycle may result in a fresh transfer of a single embryo, with less obstetric risk and thus, a higher likelihood of a term live birth. It may also yield surplus good quality embryos that can be confidently cryopreserved using vitrification and have a high likelihood of resulting in future live births, therefore not diminishing the cumulative livebirth rate. This scenario is most likely to arise in younger women with good ovarian reserve and good egg quality and thus is the reason for the recommendation for eSET in women less than 37 years old.

Vitrification was first introduced in Cork Fertility Centre in March 2012. The eSET rate in Cork Fertility Centre was 12.2% in 2013, increasing to 12.9% in 2014.

In Ireland, over the last decade (2002 - 2011), there was an increase of 52.7% in the number of liveborn twins delivered and a similar trend has been noted in other countries (162, 196, 197). Currently there is no governing body dedicated to overseeing ART in Ireland, therefore many clinics have adopted the recommendations from the UK's HFEA. Without a governing body to supervise a strict eSET policy, there continues to be high rates of multiple pregnancies in Ireland (see Table 1.6 and 1.7). The Healthcare Pricing Office and Health Service Executive in Ireland Perinatal Statistics Report 2016 demonstrates an increase in the twinning rate of 22.1 per cent over the decade 2007 - 2016 and a 1.6 per cent increase between 2015 and 2016. The lack of a governing body also results in a lack of mandatory reporting by Irish fertility clinics to help ascertain the contribution ART is making to the multiple pregnancy numbers. A varying number of the seven fertility clinics based in Ireland voluntarily report to ESHRE each year and the results of their most recent reports are shown in Table 1.7 and Table 1.8. There is a rise in the number of single embryo transfers and a consequent fall in the number of multiple embryo transfers over this time period indicating a change in practice in Irish fertility clinics in line with those adopted in other jurisdictions.

Table 1.6: Single and multiple births (both livebirth and stillbirth) in Ireland 2007-2016

Births	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	% change 2007-2016	**% change** 2015-2016
Singleton	69692	72916	73603	73046	71651	69452	66650	65070	63384	61655	-11.5	-2.7
Twin	2185	2575	2373	2480	2638	2435	2526	2461	2390	2363	8.1	-1.1
Triplet	86	96	43	74	88	99	91	79	95	79	-8.1	-16.8
Twinning Rate	15.4	17.4	15.9	16.7	18.1	17.2	18.6	18.5				

Twinning rate- per 1,000 maternities.

Data extracted from Health Service Executive, Healthcare Pricing Office, Perinatal Statistics Report 2016 (198)

Table 1.7: Irish fertility clinic data as reported to ESHRE 2008 - 2014 demonstrating numbers of embryos transferred and subsequent delivery data from IVF/ICSI embryo transfers.

Year	No. Clinics (n = 7)	No. ET[1]	1 embryo	2 embryos	3 embryos	4+ embryo	Births	Twins n (%)	Triplet n (%)
2014	3	851	349 (41)	492 (57.8)	10 (1.2)	0	331	49 (14.8)	1 (0.3)
2013	3	894	339 (37.9)	517 (57.8)	38 (4.3)	0	308	52 (16.9)	0
2012	4	1624	624 (38.4)	907 (55.8)	93 (5.7)	0	448	62 (13.8)	2 (0.4)
2011	5	1783	581 (32.6)	1047 (58.7)	154 (8.6)	1 (0.1)	472	75 (16.2)	0
2010	6	2425	679 (28)	1535 (63.3)	206 (8.5)	5 (0.2)	685	130 (19)	5 (0.7)
2009	6	2487	640 (25.7)	1639 (65.9)	208 (8.3)	0	627	137 (21.9)	6 (1.0)
2008	5	2207	422 (19.1)	1602 (72.6)	181 (8.2)	2 (0.1)	618	127 (20.6)	8 (1.3)

Data presented as n (%) where available

1- number of embryo transfers.

Table 1.8: Irish fertility clinic data as reported to ESHRE 2008 - 2014 demonstrating numbers of frozen embryos thawed and subsequent delivery data from frozen embryo transfers.

Year	No. of clinics reporting	No. of embryos thawed	Deliveries	Twins n (%)	Triplets
2014	3	385	80	7 (8.8)	0.0%
2013	3	371	77	5 (6.5)	
2012	4	716	101	9 (8.9)	0.0%
2011	5	762	124	9 (7.3)	0.0%
2010	6	882	123	13 (10.6)	0.8%
2009	6	744	114	9 (8.0)	0.0%
2008	5	672	91	11 (12.1)	1.1%

1.7 Singleton pregnancy following ART

There is evidence that ART singletons have increased perinatal risks when compared to spontaneously conceived singletons. Studies show increased rates of pre-term delivery, low birth-weight and cerebral palsy even following adjustment for factors such as maternal age and parity (176, 199). The reasons for the increased adverse outcomes are poorly understood. Many studies have suggested that the underlying subfertility may itself be a risk factor with studies showing higher rates of adverse outcomes associated with increasing duration of involuntary childlessness. The limitations of many of these studies are that subfertile women are compared to healthy fertile women (200).

Two studies adopted a sib-ship design in order to homogenise the patient population, by comparing outcomes between ART-conceived and non-ART conceived siblings- both found higher perinatal adverse outcomes in the ART-conceived sibling, in particular higher rates of pre-term delivery. The study by Romunsdstad et al found no difference after adjusted analyses, leading the authors to conclude that the subfertility, rather than the ART process, was implicated (201). The study by Aaris-Henningsen et al found a reduced but persistent increased perinatal risk following adjustment, concluding that the treatment process may also play a role in adverse outcome (202).

There have been suggestions that the embryo culture media may also play a role. One study by Kallen et al 2010 demonstrated a decline in adverse perinatal outcomes over time which may suggest that improvements in IVF techniques, including milder controlled ovarian hyperstimulation (COH), better culture media and increased eSET may have an impact.

Studies on the outcomes of pregnancies conceived from frozen embryo transfer (FET) show a lower rate of small for gestational age infants (SGA) with some studies showing an increased rate of large for gestational age (LGA) infants when compared to fresh embryo transfer (203-205). Again, the sib- ship study by Aaris-Henningsen 2011 showed that, following adjustment for age and birth order, siblings born from FET had a higher mean birthweight compared to their spontaneously conceived sibling (202). This once again suggests that the laboratory process, perhaps cryopreservation techniques, rather than maternal factors alone, may play a role.

1.8 Vanishing twin syndrome following ART

Vanishing twin syndrome (VTS) has been identified as a potential risk factor for obstetric complications, in particular pre-term delivery and low birthweight. Vanishing twin syndrome (VTS) is estimated to affect between 12 - 30% of pregnancies following ART (206). VTS is the phenomenon whereby more than one gestational sac is present on early ultrasound but due to loss of the second sac/fetus, the pregnancy continues as a singleton. Several studies have demonstrated an increased rate of adverse perinatal outcome for VTS singletons. A recent large study of 113,784 livebirths from fresh and frozen ART cycles in the UK, by Kamath et al 2018 demonstrated an increased risk of pre-term birth and low birthweight among VTS singletons when compared to singletons with only one gestational sac at early ultrasound (irrespective of whether single or double embryo transfer had occurred). These findings are consistent with a number of other prior studies (207, 208). Pinborg et al studied 8542 women and identified a 1.7-fold increased risk of low birth weight and a 2.3 - fold increased risk of very low birth weight in liveborn singletons originating from a VTS pregnancy when compared with liveborn singletons from a singleton pregnancy. The study also found that obstetric risks increased the later in pregnancy that spontaneous reduction occurred (209).

Sazonova et al performed a large population-based registry study of 13,544 children born from IVF (fresh transfer, frozen transfer, eSET, double embryo transfer, donor oocytes, singletons and multiples). Their obstetric outcomes were compared with spontaneously conceived children of the general population. This study demonstrated that IVF children, irrespective of numbers of embryos transferred or

multiplicity, had poorer obstetric outcomes, particularly in terms of pre-term delivery, low birth weight and very low birthweight (210).

1.9 Summary

Assisted reproductive technologies are now well-established treatment options for couples with subfertility with increasing numbers of couples undertaking IVF/ICSI in order to achieve a pregnancy.

The success of IVF is also improving, particularly with advancements in embryology and cryopreservation (211). This has resulted in IVF/ICSI treatment becoming more applicable to a broader population, allowing women/couples who previously may not have had the hope of conceiving such as older or menopausal women or couples with significant hereditary genetic conditions to consider pregnancy.

However, the treatment process is fraught with anxiety and stress for couples who are hoping for a much-wanted baby. Furthermore, it carries increased risks both directly and indirectly related to the process. OHSS is of particular concern and women at risk of this condition require careful management and counselling. Multiple pregnancy carries increased obstetric risk for both mother and fetuses, particularly associated with pre-term delivery, with an associated financial burden on the state in providing the necessary care. Iatrogenic twinning due to the transfer of two embryos is preventable. Therefore, appropriate patient selection for elective

single embryo transfer (eSET) should be encouraged in order to reduce the frequency of multiple pregnancy.

There is increasing focus on the physical characteristics and pre-conceptual health of the couples undergoing fertility treatment. Obesity has been shown to be associated with poorer IVF outcomes and is a risk for obstetric complications, gestational diabetes in particular and its associated complications. Smoking, alcohol and diet are also known to have negative effects on IVF treatment outcomes.

Less is known about the contribution of the physical characteristics of a normal uterus, to both treatment and pregnancy outcome. There is an indication the women with smaller uteri have a lower chance of ongoing pregnancy but the studies on this are few. There is no study regarding the combination of multiple pregnancy and smaller uteri on obstetric outcome, in particular the risk of preterm delivery, which is responsible for the majority of neonatal morbidity and mortality arising from multiple pregnancy.

1.10 Objectives

The overall objective of the thesis was to identify any pre-cycle characteristics that may indicate a negative impact on IVF success rates or increased obstetric risk. The original hypothesis was that pre-cycle characteristics, particularly uterine volume and dimensions, could be identified prior to an IVF cycle, as being associated with a higher risk of pre-term labour and adverse pregnancy outcome, therefore allowing a

more individualized embryo transfer policy *i.e.* where a woman was at lower risk for preterm delivery she could be selected for double embryo transfer. The approaches planned to fulfil the objectives are detailed in the studies outlined in sections below.

The Retrospective Study - pregnancy outcomes from ART at Cork Fertility Centre (CFC) 2002 - 2013

This retrospective review, presented in Chapter 2, was undertaken to ascertain if pregnancies (both multiple and singleton) resulting from IVF/ICSI at Cork Fertility Centre, between 2002 - 2013, were associated with adverse perinatal outcome. A more detailed study on dichorionic diamniotic (DCDA) twins (resulting from the transfer of two embryos, therefore iatrogenic), was also undertaken, comparing them to spontaneously conceived twins, with the aim of assessing whether mode of conception conferred excess perinatal risk.

The Prospective Cohort Study of nulliparous women undergoing IVF/ICSI at CFC 2012 - 2015

This aim of this prospective review, presented in Chapter 3, was to determine if any physical, biochemical, demographic, lifestyle or psychological factors that are identifiable prior to treatment may be associated with adverse treatment outcome and/or adverse obstetric outcome. Clinical Research Ethics Committee Approval was granted for this study (Appendix i and ii). No funding was sought for this study.

The Cross-sectional Study- Assessing the impact of psychological factors on treatment outcome

The aim of this study, presented in Chapter 4, was to determine if psychological stressors had an impact on IVF/ICSI treatment cycle outcomes including miscarriage rates.

Chapter 2 - The Retrospective Study

Chapter 2 – The Retrospective Study - A retrospective study of all twin and singleton pregnancies arising from IVF/ICSI treatment at Cork Fertility Centre

2.1 Methods

2.1.1 Study design

This study was a retrospective study firstly looking at all dichorionic, diamniotic (DCDA) twins resulting from IVF/ICSI treatment at Cork Fertility Centre 2002 - 2013 inclusive. DCDA twins were selected as they are iatrogenic twins i.e. as a result of the transfer of two embryos, and therefore there is potential to reduce the twin pregnancy rate by modifying embryo transfer policy. As discussed in the introduction, monochorionic twins do occur after IVF/ICSI due to splitting of a blastocyst, however they are not associated with the transfer of two embryos and therefore there is no opportunity to modify management (i.e. modify numbers of embryos transferred) to impact on monochorionic twin rates.

A further more detailed review of all DCDA twins conceived from IVF/ICSI at Cork Fertility Centre and who delivered at Cork University Maternity Hospital between 2009 - 2012 was performed. The time period for this more detailed study was selected because in 2009 the dedicated twin clinic was set up in CUMH. The twin clinic was consultant led with dedicated midwifery support. All twin pregnancies attending the clinic were prospectively documented in a dedicated database with details including method of conception (Appendix iii). This enabled the identification of cohort of spontaneously conceived DCDA twins. The outcomes of the ART

conceived DCDA twin pregnancies were compared to all spontaneously conceived DCDA twins who delivered at Cork University Maternity Hospital during the same time period in order to ascertain if mode of conception was influential on obstetric outcome.

The second group of patients studied were all singleton pregnancies resulting from IVF/ICSI treatment at Cork Fertility Centre 2002 - 2013 inclusive. Unfortunately, it was not possible to identify spontaneously conceived singleton pregnancies, who might have served as a control group, as data was not routinely or reliably collected at Cork University Maternity Hospital on mode of conception at this time. Women with twin pregnancies had mode of conception noted prospectively in the database maintained by the twin pregnancy clinic (5). However outside of the twin clinic the data was neither reliable nor easy to access without requesting the paper charts from the archives. A study we published further proved the difficulty with reliable documentation of mode of conception. The study, examined the medical charts of all oocyte donation pregnancies from Cork Fertility Centre who subsequently delivered in CUMH, during the period 2007 - 2012, and noted that only one third of women unambiguously disclosed the mode of conception of their pregnancy (212).

2.1.2 Population/participants

The groups studied as part of the retrospective study included

- all women who became pregnant with a twin pregnancy through IVF/ICSI treatment at Cork Fertility Centre and subsequently delivered at Cork University Maternity Hospital

- all women who became pregnant with a singleton pregnancy through IVF/ICSI treatment at Cork Fertility Centre

2.1.3 Data collection

The patient electronic database, IDEAS V. 5.3 ™, Mellowood Medical, Toronto Canada, was interrogated to identify those who conceived a twin or singleton pregnancy from IVF/ICSI treatment. The data is completed by fertility nurse specialists as part of the auditing of IVF/ICSI cycle outcomes. The women are asked to complete a proforma with delivery details, birthweight, mode of delivery, gender and any pregnancy complications. In the event that the woman does not send in the form, the CFC nurses contact the woman to complete the details. The data will be collected on every patient as part of the monitoring of success rates. There is frequently incomplete data on pregnancy complications, and there may be omissions on mode of delivery and birthweight, so data is not always complete (Appendix iv). Prior to 2007 the IDEAS database was not in use therefore patient logbooks were used to identify the relevant cohort of women from the period 2002 - 2006 (Appendix v). These were completed in the same manner. Maternal demographic data including age and parity were recorded. Pregnancy outcomes, including gestation at delivery, mode of delivery, birth weight and gender were identified from the patient database/logbook where available.

2.1.4 Pregnancy outcomes - DCDA twins

A detailed study of pregnancy outcomes for the DCDA twins conceived with IVF/ICSI at Cork Fertility Centre and who delivered at Cork University Maternity Hospital between 2009 - 2012 was performed. The outcomes were compared to all spontaneously conceived DCDA twins who delivered at Cork University Maternity Hospital during the same time period.

Maternal and obstetric outcomes were recorded from birth records, maternal obstetric charts and laboratory data. Specific maternal outcome measures included gestational hypertensive disorders; either pre-eclampsia (defined as persistent blood pressure measurements of ≥ 140/90mmHg after 20 weeks gestation with associated proteinuria > 300mg/24 hours) or pregnancy induced hypertension (persistent blood pressure readings ≥ 140/90mmHg, without associated proteinuria, occurring after 20 weeks gestation), gestational diabetes mellitus (diagnosed by oral glucose tolerance test at approximately 28 weeks gestation), obstetric cholestasis (pruritus and deranged liver transaminases with elevated serum bile acids), gestation at delivery, onset of labour (i.e. spontaneous or induced labour) and mode of delivery.

Perinatal outcomes were recorded from birth records, maternal obstetric charts and neonatal intensive care (NICU) database. These data were collected from BadgerNet Neonatal database (Clevermed, Edinburgh, Scotland) in NICU at CUMH. The BadgerNet Neonatal system is a contemporaneous, electronic record of events during the neonatal admission including delivery events. Perinatal outcome measures included second trimester spontaneous reduction, intrauterine fetal

demise or stillbirth of one twin (≥ 24 weeks gestation and/or weighing 500g or more), intrauterine growth restriction (IUGR; defined as estimated fetal weight less than tenth percentile for gestational age), prematurity (defined as birth occurring at less than 37 weeks gestation), degree of prematurity; extremely preterm (< 28 weeks gestation), very preterm (28 - 31^{+6} weeks gestation), moderately preterm (32 - 33^{+6} weeks gestation) and late preterm (34 - 36^{+6} weeks gestation), birth weight, low Apgar score (defined as < 5 at 1 minute and/or < 7 at 5 minutes), NICU admission and mean length of stay (days) in the NICU and a composite measure of perinatal morbidity included any of the following: hypoxic ischaemic encephalopathy (HIE), necrotising enterocolitis (NEC) and/or sepsis.

2.1.5 Pregnancy outcome - vanishing twin syndrome

A proportion of pregnancies that were confirmed as twin pregnancies on ultrasound scan at 6 and/or 8 weeks gestation resulted in loss (miscarriage, intrauterine demise (IUD)) of one twin, known as spontaneous reduction or Vanishing Twin Syndrome (VTS). VTS outcomes were compared with singleton pregnancy (those pregnancies that had a single intrauterine gestational sac, with or without a single fetal pole, on first trimester scan) outcomes.

2.1.6 Pregnancy outcome - singletons

Singleton pregnancies were defined by the presence of a single intrauterine gestational sac, with or without a single fetal pole, at first trimester ultrasound scan.

The pregnancy outcomes for the singleton pregnancies conceived by IVF/ICSI included the following pregnancy- biochemical pregnancy, first trimester miscarriage, second trimester miscarriage, intrauterine fetal demise, neonatal death, live birth, gestation at delivery, mode of delivery and gender and birth weight of neonate

2.2 Results

During the defined study period for the retrospective cohort 2,456 pregnancies resulted from IVF/ICSI treatment. 13.4% (n = 331) of positive pregnancy tests resulted in a biochemical pregnancy. 73% (n = 1791) were singleton pregnancies and 13.6% (n = 334) were twin pregnancies, as defined on ultrasound scan at 6 or 8 weeks gestation.

2.2.1 Maternal characteristics

The women were studied according to age, parity, previous ART and pregnancy outcome. There was no difference between the women who had a biochemical pregnancy and those that continued pregnancy. As regards those women who had previous assisted reproductive treatment other than IVF/ICSI there was no difference in the numbers of women who conceived singletons or twins (67.4% versus 61.8%). Similarly, there was no difference in the rates of those who had a previous pregnancy (miscarriage) from IVF/ICSI for mothers who conceived singletons or twins (26.8% versus 20.2%). On average, women who conceived twins

were older than those who conceived singletons (36.2 years, SD ± 3.4 years versus 35.3 years, SD ± 3.8 years).

2.2.2 Singleton pregnancy outcomes

As displayed in Table 2.1, 80.2% (n = 1435/1790) of singleton pregnancies resulted in a livebirth. There were 4 neonatal deaths (0.3%; n = 4/1791). The rate of early miscarriage was 18.9% (n = 340/1790) (Table 2.1).

Over half of singletons were delivered by vaginal delivery (54.3%; n = 710/1306). One third (29.9%; n = 390/1306) were delivered by elective caesarean section and the remainder required emergency caesarean section (15.8%; n = 206/1306). The vast majority of singleton pregnancies were delivered at term (93.4%; n = 1297/1389), 5% (n = 70/1389) were delivered at late pre-term gestation (34 - 36+6 weeks gestation). Delivery data was incomplete for n = 129 singleton pregnancies.

2.2.3 Twin pregnancy outcomes

There were 291 twin pregnancies, of which 86.8% resulted in a livebirth of one or both twins (n = 449 liveborn babies). Almost half of all twin pregnancies resulted in a livebirth of both twins (47.2%; n = 158/335). However, two of the liveborn twin pairs experienced a neonatal death of one twin (Table 2.1).

39.7% (n = 133/335) of twin pregnancies resulted in the livebirth of only one twin. Spontaneous reduction (vanishing twin syndrome, VTS) of one twin occurred most frequently due to early miscarriage (37.6%; n = 127/335) (Table 2.1).

Only 13.4% (n = 45/335) of twin pregnancies did not result in a livebirth, most frequently due to early miscarriage of both twins (11.1%; n = 37/335). One twin pregnancy resulted in intrauterine demise of both twins (Table 2.1). The majority, 67.2% (n = 300/448) of liveborn twin infants were born at term gestation (≥ 37 weeks gestation). A further 20.8% (n = 93/448) liveborn twin infants were born at late pre-term gestation (34 - 36+6 weeks gestation). The range of gestations at birth was 41+6 weeks gestation – 25 weeks gestation for liveborn twin infants.

Gestation at delivery was analysed according to infants where both infants of a twin pair were liveborn to those infants that started out as confirmed twin pregnancies but spontaneously reduced to liveborn singleton (Vanishing Twin Syndrome; VTS). VTS twins were significantly more likely to result in a term delivery (92.4%; n = 122/132 v 56.5%; n = 179/318, p = 0.000). The gestational age range of spontaneously reduced twins was 41+6 – 25 weeks gestation compared with a gestational age range of 39+4 - 25+2 weeks gestation for infants of a twin pair where both twins were liveborn.

Twins were most commonly delivered by elective caesarean section (37.3%; n = 162/434 babies) and 30.9% (n = 134/434) being delivered by vaginal delivery (data was incomplete on the mode of delivery of 15 babies). However, when the figures are divided into both babies liveborn versus livebirth of only one infant (VTS twin

pregnancy) the latter are significantly more likely to be delivered vaginally (42.5%; n = 51/120 v 26.4%; n = 83/314, p = 0.003).

In terms of birthweight, the median birthweight for twins was 2495g ± 645.7g with twin one having a significantly higher birthweight than twin two (2889g SD ± 688g versus 2467g SD ± 575g). Twin two was more likely to be admitted to NNU than Twin 1 (38.3% versus 29.2%; p = 0.54).

2.2.4 Singleton pregnancy outcomes versus twin pregnancy outcomes

Pregnancy outcomes of singleton pregnancies versus twin pregnancy were analysed and presented in Table 2.2. Singleton pregnancies were more likely to result in a livebirth while twin pregnancies were more likely to result in miscarriage (one or both twins - 34.4% versus 19.4% miscarriage; p = 0.000).

Twins were four times more likely to be delivered before 36 weeks gestation (27.1% versus 6.5%; p = 0.000) Twins are more likely to be admitted to the NNU than singletons (32.6% versus 11.7%; p = 0.000).

Singleton infants had a significantly higher birthweight than twin infants (3422g ± 573g versus 2739g ± 680g)

2.2.5 Singleton pregnancy outcomes versus vanishing twin syndrome (VTS) singleton outcomes

A proportion of pregnancies that were confirmed as twin pregnancies on ultrasound scan at 6- or 8-weeks gestation resulted in loss (miscarriage, IUD) of one twin, known as spontaneous reduction or Vanishing Twin Syndrome (VTS). VTS singleton outcomes were compared with singleton outcomes (Table 2.2).

There was no significant difference in gestation at delivery between the two groups (p = .670). VTS singletons were significantly more likely to be delivered by emergency caesarean section (24.2%; n = 29/120 v 15.8%; n = 206/1307; p = 0.018). There was no significant difference in birthweights between the two groups (3423g SD ± 573g v 3387g SD ± 525g; p = .493). There was no difference in the rate of admission to NNU (107.7%; n = 130/1114 v 12%; n = 15/125, p = .967) (Table 2.2).

Table 2.1: Outcomes of all pregnancies from IVF/ICSI treatment at Cork Fertility Centre 2002 - 2013 inclusive

	Number of pregnancies (n = 2457)	**Number of infants**
Biochemical pregnancies	331 (13.4%)	
Singleton pregnancies	1790 (72.8%)	
Livebirth	1435 (80.2%)	1435
Livebirth but NND[1]	4 (0.3%)	
No livebirth	355 (19.8%)	
Early miscarriage	341 (18.9%)	
Late miscarriage	8 (0.4%)	
IUD[2]	7 (0.39%)	
Twin pregnancies	335 (13.6%)	449
Livebirth both twins	158 (47.2%)	316
Livebirth both twins but early NND[2] one twin	2 (0.6%)	
Livebirth both twins but late NND[1] one twin	1 (0.3%)	
Livebirth one twin (vanishing twin syndrome)	133 (39.7%)	133
Livebirth/early miscarriage	126 (37.6%)	
Livebirth/late miscarriage	5 (1.5%)	
Early miscarriage one twin and early NND[1] of second twin	2 (0.6%)	
No livebirth	45 (13.4%)	
IUD[2] both twins	1 (0.3%)	
Early miscarriage both twins	37 (11.1%)	
Early miscarriage and late miscarriage	1 (0.3%)	
Late miscarriage and ectopic pregnancy	1 (0.3%)	
Late miscarriage both twins	5 (1.5%)	

1 - NND - neonatal death, 2 - IUD - intrauterine fetal demise

Table 2.2: Outcome of singleton and twin pregnancies, including vanishing twin syndrome (VTS) singletons.

Outcomes	**Twin pregnancies (n = 335 pregnancies)**	**Both twins ongoing (n = 318 infants)**	**VTS[1] singletons (n = 133)**	**Singleton pregnancies (n = 1790)**
Livebirth	291 (86.8%)	158	133	1435 (80.16)
Livebirth (infants)	449	316	133	1435
Mode of delivery (infants)				
Vaginal delivery	134/434 (30.9)	83/314 (26.4)	51/120 (42.5) * (p = .003 v both twins)	710/1307 (54.3) * (p = 0.018 v VTS)
Elective caesarean	162/434 (37.3)	122/314 (38.9)	40/120 (33.3)	391/1307 (29.9)
Emergency caesarean	138/434 (31.8)	109/314 (34.57)	29/120 (24.2)	206/1307 (15.8)
Gestation at delivery (infants)				
> 37 weeks	301/448 (67.2)	179/316 (56.6) *	122/133 (93.1)	1297/1389 (93.4)
34 – 36 + 6 weeks	93/448 (20.8)	87/316 (27.5)	6/133 (4.6)	70 (5.0)
32 – 33 + 6 weeks	24/448 (3.6)	22/316 (7)	2/133 (1.5)	8 (0.6)
28 – 31 + 6 weeks	23/448 (3.4)	22/316 (7)	1/133 (0.8)	12 (0.9)
24 – 27 + 6 weeks	7/448 (1.0)	6/316 (1.9)	1/133 (0.8)	2 (0.1)
Admission to NNU[2] (infants)	140/430 (32.6)	127/308 (41.2)	15/125 (12.0)	130/1114 (11.7)
Perinatal mortality rate (IUD[3]/NND[2])	6/449 (13.3)	4/316 (12.7)	2/133 (15.0)	11/1435 (7.66)
NND[2] (infants)	4	2	2	4
IUD[3] (infants)	2	2	0	7

Data presented as n (%). 1 - VTS - Vanishing Twin Syndrome, 2 - NND - neonatal death, 3 - IUD - intrauterine fetal demise

2.3 Detailed study of DCDA twin outcomes comparing both spontaneous conception and IVF/ICSI

The pregnancy outcomes of all DCDA twin pregnancies conceived with IVF/ICSI at Cork Fertility Centre and subsequently delivered at Cork University Maternity Hospital (CUMH) during the period 2009 - 2012 were analysed. The results were compared with the outcomes of all spontaneously conceived DCDA twin pregnancies delivered at CUMH during the same period.

2.3.1 Mode of conception

Five hundred and forty-four women were identified as have an ongoing DCDA twin pregnancy at approximately 12 weeks gestation. One hundred and seventy-one women (31.7%) had conceived using assisted reproductive technologies (ART). One hundred and nine women (63.7%) conceived following IVF treatment, 20.5% (n = 35) following ICSI treatment and 15.8% (n = 27) had IVF/ICSI using donor oocyte.

2.3.2 Maternal characteristics

The mean maternal age of the women was 33.7 ± 5.17 years. Women who conceived twins with IVF/ICSI were significantly older 36.8 ± 4.23 years v 32.3 ± 4.93 years ($p < 0.001$). Women conceiving with IVF/ICSI were significantly more likely to be nulliparous compared to those who spontaneously conceived twins 73.7%; n = 126 v 36.1%; n = 133 ($p < 0.001$) (Table 2.3).

2.3.3 Maternal outcomes

There was no significant difference in the frequency of maternal antenatal complications between the two groups, specifically gestational diabetes mellitus, gestational hypertensive disorders (PET or PIH) and obstetric cholestasis. There was a slightly higher rate of gestational hypertensive disorders in the IVF/ICSI group compared with the spontaneously conceived group, however it did not reach statistical significance and when adjusted for age, parity and type of antenatal care (public v private), there was no significant difference between the groups (Table 2.3).

Table 2.3: Maternal characteristics of IVF/ICSI versus spontaneously conceived DCDA twin pregnancies

Maternal characteristics	Total population n = 539	Spontaneously conceived n = 368	IVF/ICSI n = 171	Unadj. OR[1] (95% CI)	Adj. OR[2] (95% CI)	P - value
Mean age (years ± SD)	33.7 ± 5.2	32.3 ± 4.9	36.8 ± 4.2	-	-	-
Age range (years)	17 - 51	17 - 46	26 - 51	-	-	-
Nulliparity	259 (48.1)	133 (36.1)	126 (73.7)	-	-	< 0.001
Multiparity	280 (51.9)	235 (63.9)	45 (26.3)	-	-	< 0.001
Antenatal complications[3]						
- GDM[4]	22 (4.1)	14 (3.8)	8 (4.7)	1.24 (0.66 - 1.81)	0.86 (0.41 - 1.81)	0.709
- GHD[5]	100 (18.6)	63 (17.1)	37 (21.6)	1.34 (0.97 - 1.84)	0.81 (0.55 - 1.20)	0.128
- OC[6]	21 (3.9)	12 (3.3)	9 (5.3)	1.65 (0.88 - 3.08)	1.39 (0.66 - 2.95)	0.188

Data presented as n (%) where applicable and unless otherwise stated

1 Unadj. OR unadjusted odds ratio

2 Adj. OR - adjusted odd ratio

3 Antenatal cx - antenatal complications

4 GDM - gestational diabetes,

5 GHD - gestational hypertensive disease,

6 OC= obstetric cholestasis

2.3.4 Delivery

Induction rates were similar between the groups, (29.2%; n = 214 spontaneously conceived group v 24.9%; n = 85 IVF/ICSI group), however spontaneously conceived pregnancies were more likely to spontaneously labour than IVF/ICSI twins (31.4%; n = 230 spontaneously conceived v 20.5%; n = 70 IVF/ICSI; p < 0.001) this is likely due to the higher rate of caesarean delivery (both elective caesarean and emergency caesarean before labour) amongst the IVF/ICSI group. (Table 2.4) IVF/ICSI twins were twice as likely to be delivered by caesarean section (CS) than those conceived spontaneously (OR 2.35; 95% CI 1.76 - 3.14). This association remained after adjustment for confounding variables, specifically maternal age, parity and type of antenatal care (AOR 1.45 95% CI (1.03 - 2.03). IVF/ICSI twins were more likely to be delivered by emergency CS (OR 2.47, 95% CI 1.78 - 3.44).

Almost half (49.7%; n = 536) of all the twin pregnancies in the study group were delivered before 37 weeks gestation, most frequently (44.6%; n = 239) following spontaneous onset of labour. There was no difference in the rates of preterm birth when mode of conception was considered (OR 0.95, 95% CI 0.73 - 1.22, p = 0.879) and following adjustment for maternal age, parity and type of antenatal care (AOR 0.92, 95% CI 0.68 - 1.28). There was a significantly higher rate of spontaneous preterm labour in spontaneously conceived group (49.1% v 34.7%; p < 0.001).

The cause of preterm birth was reviewed for each conception group (spontaneous onset of labour, induction of labour, elective caesarean section or

emergency caesarean section before labour) and is shown in Table 2.4. Almost one-third (29.9%; n = 50) of ART conceived twins were delivered by pre- labour emergency caesarean section compared with 15.7% (n = 58) of spontaneously conceived twins ($p < 0.001$).

Only 1.5% of babies were delivered at extreme prematurity (< 28 weeks gestation), with similar distribution across the spontaneously conceived and IVF/ICSI groups (1.6% and 1.2% respectively, $p = 0.433$) (Table 2.4). IVF/ICSI conceived twins were almost twice as likely to be delivered moderately preterm (32 - 33^{+6} weeks gestation) when compared to spontaneously conceived twins (OR 1.98, 95% CI 1.21 - 3.23). However, when adjusted for maternal age, parity and type of antenatal care, the significance did not remain (AOR 1.63, 95% CI .914 - 2.90).

Table 2.4: Preterm delivery

	Total population (n = 536) *	**Spontaneously conceived (n = 369) ***	**ART conceived (n = 167) ***	**Odds Ratio (95% CI)**	**p-value**
Preterm delivery	536 (49.7)	369 (50.1)	167 (48.8)	0.95 (0.73 - 1.22)	0.879
Mode of preterm delivery					< 0.001
Spontaneous onset of labour	239 (44.6)	181 (49.1)	58 (34.7)		
Induction of labour	73 (13.6)	56 (15.2)	17 (10.2)		
Elective CS[1]	116 (21.6)	74 (20.0)	42 (25.1)		
Emergency CS (pre- labour)	108 (20.1)	58 (15.7)	50 (29.9)		
Gestation at delivery					
≥ 37	538 (50.1)	364 (49.7)	174 (51%)	.784 (.586 - 1.04)	
34 - 36^{+6}	385 (35.8)	280 (38.2)	105 (30.8)	1.98 (1.2 - 3.2)	
32 - 33^{+6}	74 (6.9)	38 (5.2)	36 (10.6)	1.18 (.679-2.05)	
28 - 31^{+6}	61 (5.7)	39 (5.3)	22 (6.5)	.697 (.222 - 2.1)	
< 28 weeks	16 (1.5)	12 (1.6)	4 (1.2)		

*Values are shown as n (%), where n = number of babies delivered

1 CS - caesarean section

2.3.5 Perinatal outcomes

Perinatal outcomes were similar between the two groups and are shown in Table 2.5. There was no difference in the rate of antenatally diagnosed intrauterine growth restriction or in the rate of LBW and VLBW babies according to mode of conception. A small percentage of twins (3.6%) had a low Apgar score at birth with no significant difference according to mode of conception ($p = 0.414$). Mode of conception had no impact on the rate of perinatal death (Table 2.5).

Of those babies born alive, admission to the NICU occurred in 39.4% of cases, with 39% of IVF/ICSI twins and 40.2% of spontaneously conceived twins requiring admission ($p = 0.384$) (Table 2.5). The most frequent indication for admission to NICU was prematurity (74.9% of admissions), followed by LBW (51.8%) and RDS (31.4%). IVF/ICSI twins were more frequently admitted to Neonatal Intensive Care Unit (NICU) for reasons of LBW and for infection risk. A composite measure of neonatal morbidity showed no difference between the two groups. IVF/ICSI conceived twins were more likely to have neonatal hypoglycaemia and respiratory distress syndrome (RDS) than spontaneously conceived twins ($p < 0.05$) and the association remained when adjusted for pre-term delivery (Table 2.5).

Table 2.5: Perinatal Outcomes

	Total population (n = 1,078)*	**Spontaneously conceived (n = 736)***	**ART conceived (n = 342)***	**p-value**
IUGR	135 (12.5)	**98 (13.3)**	**37 (10.8)**	**0.146**
Low Apgar score[1]	38 (3.6)	25 (3.4)	13 (3.9)	0.414
Mean birth weight:				
Twin 1 (g ±SD)	2492.2	2501.7 ± 540.2	2471.8 ± 557.9	0.554
Twin 2 (g ±SD)	2461.5	2466.2 ± 552.6	2451.6 ± 559.6	0.777
LBW[2]	434 (40.6)	292 (40.1)	142 (41.6)	0.474
VLBW[3]	67 (6.3)	42 (5.8)	25 (7.3)	0.474
Fetal anomaly[4]	32 (3.8)	24 (3.3)	8 (2.3)	0.267
Perinatal death[5]	9 (0.8)	8 (1.1)	1 (0.3)	0.166
IUFD[6]	2 (0.2)	2 (0.3)	0	0.466
NND[7]	7 (0.7)	6 (0.8)	1 (0.3)	0.293
NICU admission	423 (39.4)	286 (39)	137 (40.2)	0.384
Mean length of stay NICU (days ±SD)	18.4 ± 19.9	18.16 ± 20.6	18.9 ± 18.4	0.704
Neonatal morbidity (Composite outcome[8])	69 (16.3)	50 (17.5)	19 (13.9)	0.347
Hypoglycaemia	25 (5.9)	10 (3.5)	15 (10.9)	< .005
RDS[9]	91 (21.5)	50 (17.5)	41 (29.9)	< .005

* values are shown as n (%) unless otherwise stated

1 Low Apgar score defined as Apgar score of < 5[1] and/or < 7[5]

2 Low Birth Weight (< 2500g)

3 Very low birth weight (< 1500g)

4 Fetal anomaly includes all anomalies except patent ductus arteriosus (PDA) in a premature infant

5 Perinatal Death - death of a fetus at weighing ≥ 500g and /or at ≥ 24 weeks gestation, or death of an infant between birth and the end of the neonatal period.

6 Intrauterine fetal demise

7 Neonatal death

8 Composite outcome includes hypoxic ischaemic encephalopathy (HIE), necrotising enterocolitis (NEC) and/or sepsis.

9 RDS- respiratory distress syndrome

2.4 Summary

Both singletons and twins conceived from IVF/ICSI had high rates of livebirth at term. Twins pregnancies resulted in livebirth of both twins in almost half of all pregnancies. Over one-third of twin pregnancies resulted in the livebirth of one twin due to VTS and most frequently caused by first trimester miscarriage of one twin. VTS singletons were significantly more likely to be delivered at term gestations and/or by vaginal delivery than pregnancies that continued as twins. When compared to true singleton pregnancies, gestation at delivery was similar but VTS singletons had a significantly higher rate of caesarean delivery.

Twins conceived following IVF/ICSI had a higher rate of caesarean delivery and a higher rate of delivery at moderately pre-term gestations. There was no difference in perinatal mortality. Associated with this there was a higher rate of RDS and neonatal hypoglycaemia. Maternal outcomes were similar with no increased risk associated with mode of conception. Overall, twin outcomes were more favourable than initially hypothesised, particularly the gestations at delivery, with 1.5% of infants (irrespective of mode of conception) being delivered at extreme prematurity.

Chapter 3 - Prospective Cohort Study

Chapter 3 - The Prospective Cohort Study - A prospective study of nulliparous women attending for IVF/ICSI treatment at Cork Fertility Centre.

3.1 Methods

A prospective study of nulliparous women attending for IVF/ICSI treatment at Cork Fertility Centre.

3.1.1 Population

All nulliparous women attending for IVF/ICSI treatment at Cork Fertility Centre were eligible for recruitment. Exclusion criteria were multiparity, previous preterm delivery (spontaneous delivery prior to 37 weeks gestation) or late miscarriage (pregnancy loss between 12 + 1 and 23 + 6 weeks gestation), a history of cervical excisional surgery (Large Loop Excision of the Transformation Zone (LLETZ) or knife conisation), pre-existing serious maternal medical disorders that may increase risk of pre-term delivery (e.g. essential hypertension, poorly controlled diabetes, epilepsy), the use of confounding treatment (e.g. elective cervical suture placement). BMI was calculated using the formula and measured according to kg/m^2. Cork Fertility Centre had a policy not to allow women with BMI ≥ $38kg/m^2$ to proceed with ART.

3.1.2 Recruitment

I performed the recruitment and, in my absence, it was performed by CFC fertility nurses Margaret O'Donnell, Brid O'Keeffe or Patricia O'Regan RGN. Women were selected for recruitment at their appointment for ultrasound scan following the downregulation phase of their treatment cycle, if using an agonist protocol, or at their baseline ultrasound scan if using an antagonist protocol. The study was explained to the woman and if they agreed to participate in the study a short proforma was completed, documenting pertinent data. A consent form was signed (for sample consent form see appendix vii). Transvaginal ultrasound was performed in order to capture 2D and 3D images of the uterus for later measurement. Bloods for biochemical markers were routinely performed as part of the work up for the IVF/ICSI cycle (see appendix vi)

3.1.3 Ultrasound measurement of uterine dimensions

All women underwent both a 2-dimensional (2D) and 3-dimensional (3D) ultrasound of the uterus as part of the recruitment process using a Voluson™ E8., GE Healthcare, US. All images were saved by the operator in a database and according to the woman's study identification number in order that volume measurements could be performed.

In the 2D image the following measurements were performed; length of the uterus measured from external os to fundus (serosal surface), length of uterus measured from the external os to the fundus of the endometrial cavity (identified as

the uppermost portion of the endometrium at the endometrial/myometrial junction) and cervical length, measured from external os to internal os. Cervical length in a non-gravid uterus is difficult to accurately measure given the absence of the amniotic fluid-filled gestational sac which serves as a clear marker of the junction between the internal cervical os and the endometrial cavity. During the literature review, several studies were noted to have a description of cervical length measurement in a non-gravid uterus, all using an estimation of internal cervical os according to ultrasound appearance. Gentry et al 2000 described the cervical length measurement as follows 'The length of the cervix was measured to the nearest 0.1 cm in a straight line from the internal os along the cervical canal to the external os. Typically, three measurements were made and the measurements that most closely demonstrated the appropriate landmarks were used', with another study referencing this method also. (213) (214) The study by Carcopino et al (BJOG 2012) on cervical length post-LLETZ treatment determined the location of internal cervical os 'the upper limit of the cervix was arbitrarily defined as a plane perpendicular to the cervical canal positioned at the inferior limit of the endometrial line' (213, 215).

A 3D ultrasound image was performed using 3D transvaginal ultrasound probe. The images were stored and volume analysis was performed using GE Healthcare software 4D view. Uterine volume measurement was performed using VOCAL II volume analysis, manually measuring uterine contour in 9° rotations. The contour measured was the serosal surface of the uterus including cervical canal.

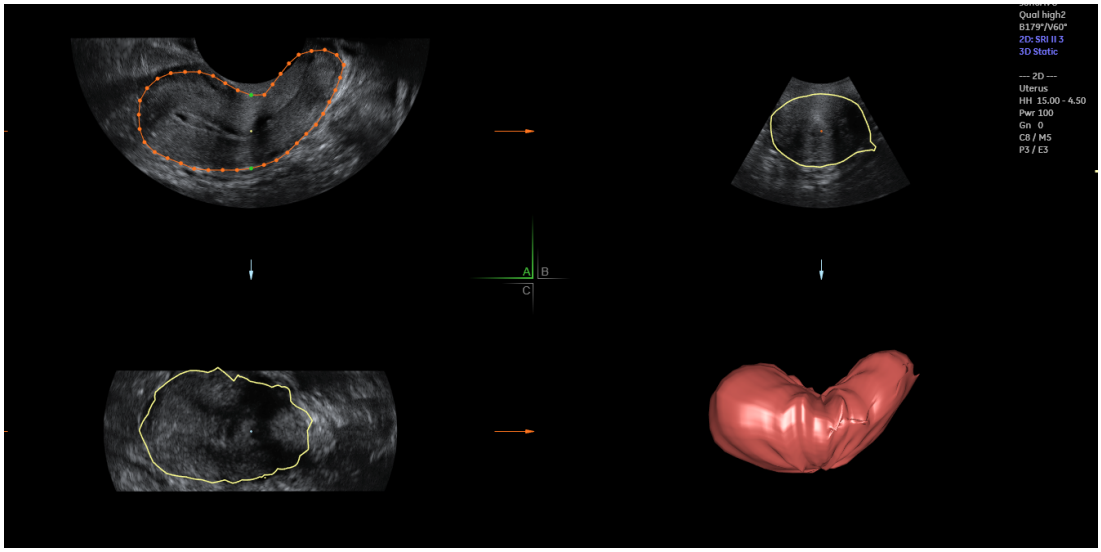

Image 3.1: Sample image of volume analysis report 4D view, GE Healthcare

3.1.4 Follow up of treatment and pregnancy outcomes

All ongoing pregnancies were analysed for the occurrence of obstetric complications. These included gestational diabetes, pre-eclampsia, pregnancy induced hypertension, intrauterine growth restriction, pre-term pre-labour rupture of membranes, oligohydramnios, placenta praevia, placental abruption and any fetal abnormalities including chromosomal and structural.

3.2 Patient characteristics

142 women were recruited for this study according to the inclusion criteria, with a mean age of 35.27 ± 3.42. Their partners were older by 3 years on average (37.1 years ± 6.077). The majority of respondents (85%; n = 121/142 women and 82.4%; n = 117/142 men) were white Irish. Over one third of both male and female partners had attended university. Over two thirds of the cohort had a normal body mass index (BMI) (18.5 - 24.9 kg/m^2) and a further one third were overweight (25 -

29.9 kg/m^2). Almost all women (90%; n = 122) were non-smokers but over three-quarters drank alcohol, the majority drinking up to 5 units per week (Table 3.1, 3.2).

All women were nulliparous, one quarter had a history of first-trimester miscarriage, the majority of whom (63.8%; n = 23/36) had conceived spontaneously. Almost half of women had undergone other forms of ART- ovulation induction and/or intrauterine insemination- prior to IVF treatment (Table 3.2).

Table 3.1: Demographics of recruited women and their partners

Demographic	**Woman**	**Partner**
Age (year ± SD)	35.27 ± 3.42	37.1 ± 6.077
Range (years)	27 - 43	26 - 50
Ethnicity		
White Irish	121 (85.2)	117 (82.4)
Irish traveller	1 (0.7)	1 (0.7)
Any other white	17 (12)	22 (15.5)
Black/black Irish	1 (0.7)	1 (0.7)
Asian	0 (0)	0 (0)
Mixed ethnicity	1 (0.7)	0 (0)
Other	1 (0.7)	0 (0)
Education (highest completed)		
University	53 (37.3)	49 (35)
College	41 (28.9)	37 (26.4)
2nd level	37 (26.1)	41 (29.3)
Primary level	0 (0)	4 (29)
Diploma/cert	11 (7.7)	8 (5.7)

Data presented as n (%)

Table 3.2: Characteristics and lifestyle factors of women recruited

Characteristic	**Women (n = 142)**
BMI	
Underweight (< 18.5)	3 (2.1)
Normal (18.5 - 24.9)	90 (63.8)
Overweight (25 - 29.9)	41 (28.9)
Obese (30 - 34.9)	6 (4.2)
Obese Cat 2 (34 - 39.9)	0 (0)
Obese Cat 3 (≥ 40)	1 (0.7)
Smoker Yes	16 (11.3)
< 5 cigarettes/day	6 (4.2)
5 - 10 cigarettes/day	4 (2.8)
11 - 20 cigarettes/day	6 (4.2)
Alcohol Yes	111 (78.2)
1 - 2 units/week	53 (47.7)
3 - 5 units/week	39 (35.1)
6 - 10	19 (17.1)
Previous miscarriage	36 (25.4)
Spontaneously conceived	23 (16.9)
ART conceived	13 (9.2)

Data presented as n (%)

3.3 Investigations

The majority of women (74.8%, n = 101/135) had an appropriate thyroid stimulating hormone (TSH) level (≤ 2.5 mIU/l). A small number had high TSH levels > 4 mIU/L (5.9%; n = 8/135).

As regards biochemical assessment of ovarian reserve the majority of women had a normal FSH level, indicating normal ovarian function (85.5%; n = 118/142) (Figure 3.1). Anti-Müllerian hormone (AMH) levels were performed on 117 women (82.3% of the population) as an indicator of ovarian reserve - decreasing levels of AMH indicate an increasingly poor ovarian reserve. Almost two-thirds had a low AMH level < 15pmol/L (64.1%; n = 75/117) and 12.8% (n = 15/117) had an extremely low AMH level (< 5 pmol/L) (Figure 3.2).

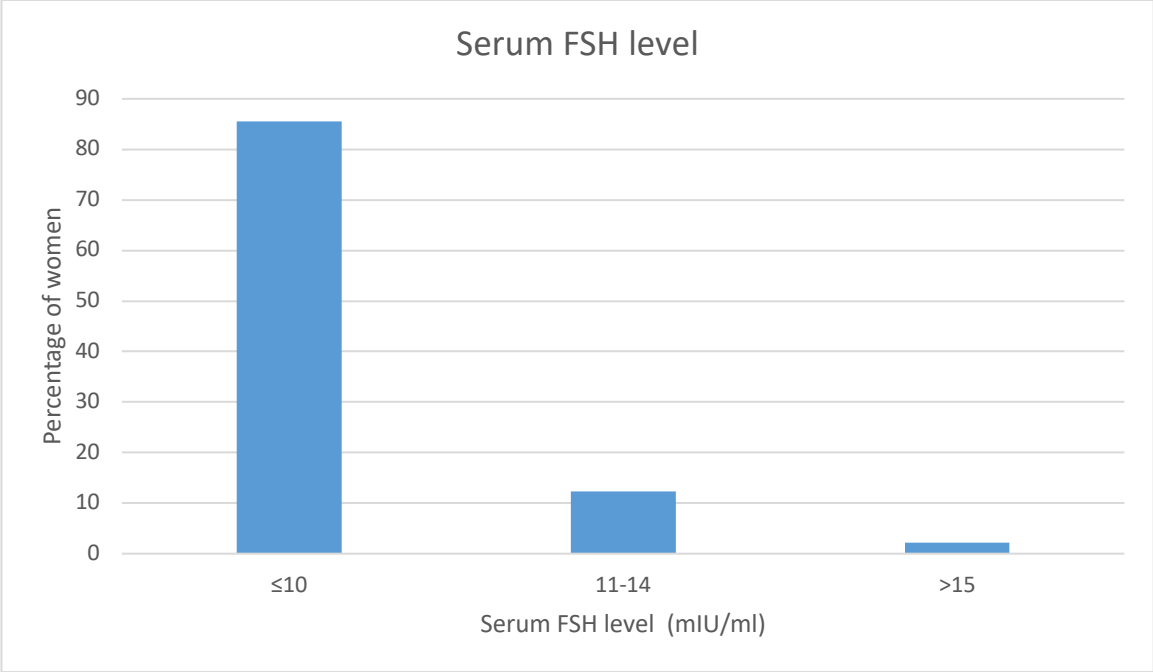

Figure 3.1: Serum Follicle Stimulating Hormone (FSH) level (mIU/ml)

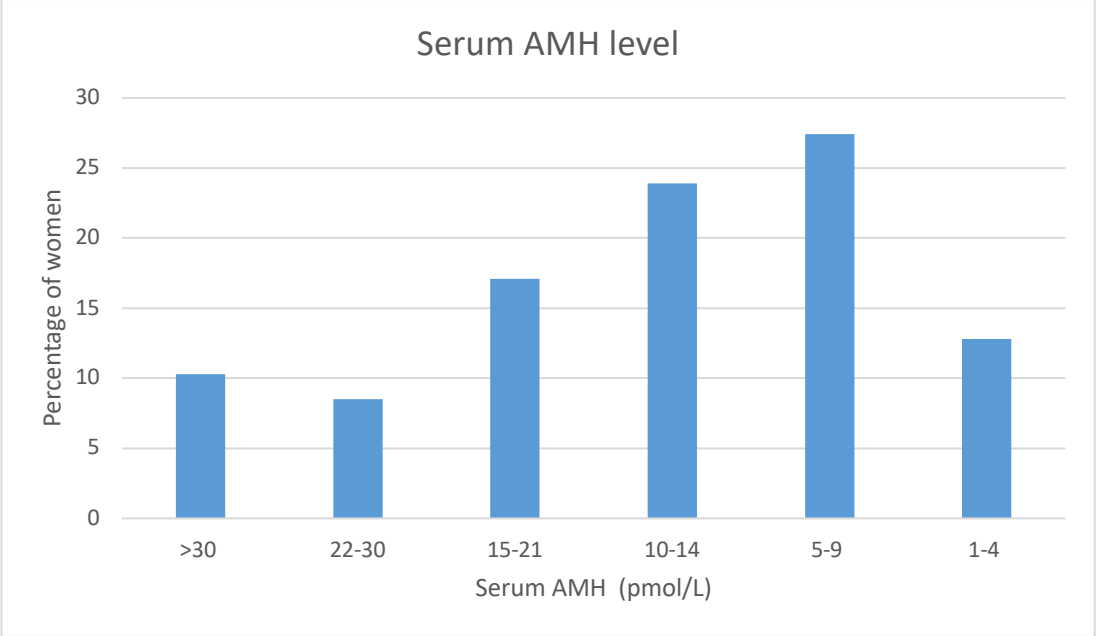

Figure 3.2: Serum Anti-Mullerian hormone (AMH) level (pmol/L)

3.4 Ultrasound assessment

Transvaginal ultrasound is used as another tool to assess ovarian reserve. The number of antral follicles visible on each ovary are calculated to give an estimate of ovarian reserve (216). Again, the majority (59.8%; n = 85/142) had a combined antral follicle count below the normal range. A small number had an extremely low combined antral follicle count (4.2%; n = 6/142) (Figure 3.3).

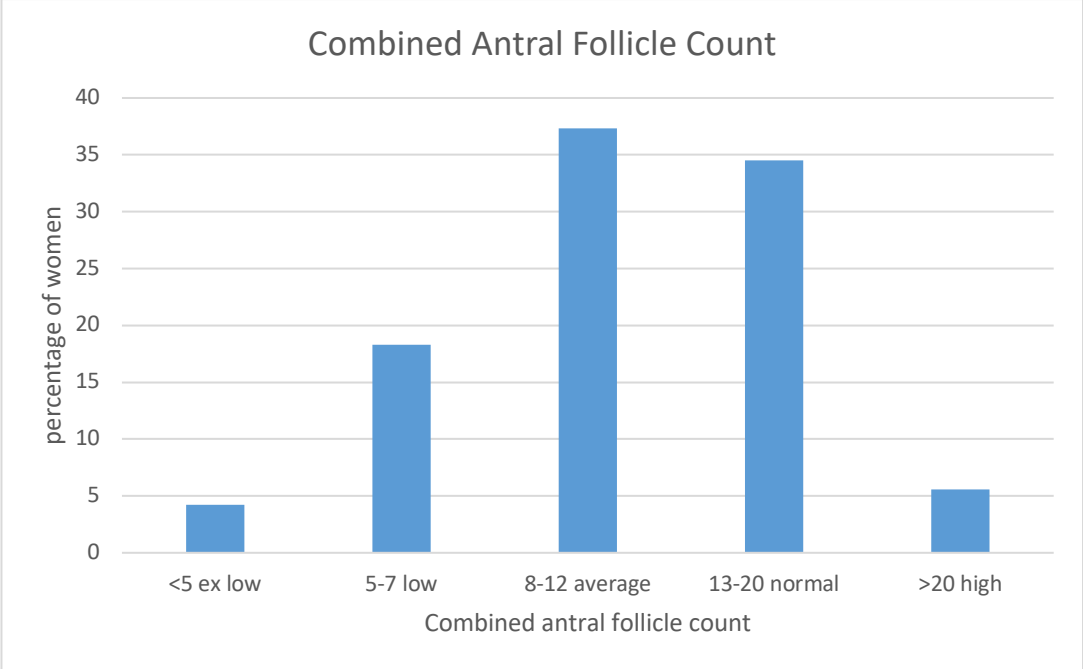

Figure 3.3: Combined Antral Follicle Count (AFC) at transvaginal ultrasound

3.5 Aetiology of subfertility

The most frequent cause of subfertility among the study cohort was diminished ovarian reserve (DOR) (38.7%; n = 55/142), with unexplained subfertility and male factor as the next most common aetiologies as shown in Table 3.1. Thirteen patients had two factors involved in their subfertility, most frequently was male factor (n = 9) but tubal factor (n = 1) and endometriosis (n = 3) was also noted (Table 3.3).

Table 3.3: Aetiology of subfertility

Aetiology	Number	Percentage
Unexplained	30	21.1
Diminished ovarian reserve	55	38.7
Tubal factor	17	12
Male factor	30	21.1
Endometriosis	4	2.8
Ovulatory dysfunction	5	3.5
Same sex (donor sperm)	1	0.7
Dual factor	13	9.2

Dual factor= both male and female partner aetiology

3.6 Uterine characteristics

Transvaginal ultrasound was used to measure uterine dimensions and 3D ultrasound technology was incorporated to measure uterine volume.

The mean length of the uterus measured from the external os to serosal surface of the fundus of the uterus was 7.26cm ± 0.88cm. The mean uterine volume was 57.33cm^3 ± 20.68cm^3.

Uterine volumes and uterine length (external os to fundus) were categorised into three groups based on 30th and 70th centile measurements. Small uterus (< 47 cm^3), average uterus (47 - 60cm^3), large uterus (> 60cm^3). For external os to fundus measurement: small cavity < 7cm, average cavity 7 - 7.6cm and large cavity > 7.6cm (Table 3.4).

Table 3.4: Uterine volume and length of uterus divided according to the 30th and 70th centiles of measurements

Uterine dimensions	Number of patients	Percentage
Volume		
< 47 cm^3	70	33.8
47 - 60 cm^3	68	32.9
> 60 cm^3	69	33.3
Length of uterus (external os to fundus)		
< 7 cm	65	34.6
7 - 7.6 cm	55	29.3
> 7.6 cm	68	36.2

3.7 Cycle characteristics

Two thirds of women required IVF treatment (65.7%; n = 92/140) with the remainder having ICSI treatment (34.2%; n = 38/140), with three of the male partners requiring testicular sperm extraction (TESE).

The median number of oocytes retrieved at oocyte collection, over all cycles, was 9 with a range of 1 - 42 eggs. Almost three quarters of embryo transfers were of two embryos (72.1%; n = 147/204) and most frequently occurring on day 5 (56.9%; n = 62). In three cases three embryos were transferred but none of the women became pregnant. The number of embryos transferred per cycle is displayed below in figure 3.4. Consistently, there is a high rate of double embryo transfer.

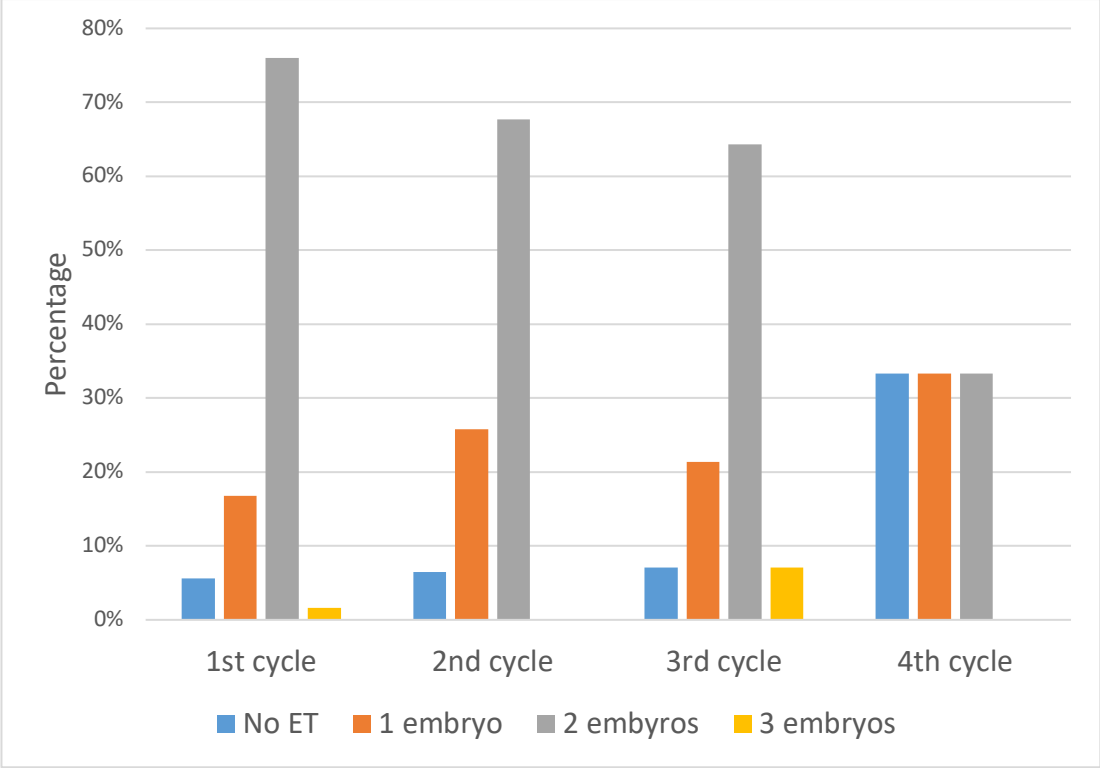

Figure 3.4: The number of embryos transferred according to cycle number

Almost half (47.2%; n = 67/142) of the women who commenced an IVF cycle had a positive HCG in their first cycle. Over all cycles the rate of positive HCG is shown in Figure 3.5.

The results are broken down as follows:

- 'per cycle started' - includes all women who initiated an IVF/ICSI treatment cycle
- 'per egg collection' - includes those who achieved egg collection (this omits those women who had their cycle cancelled before egg collection, e.g. poor response to stimulation)
- 'per embryo transfer' - omits those who did not have an embryo available for transfer - e.g. those who had failed fertilisation, poor embryo development

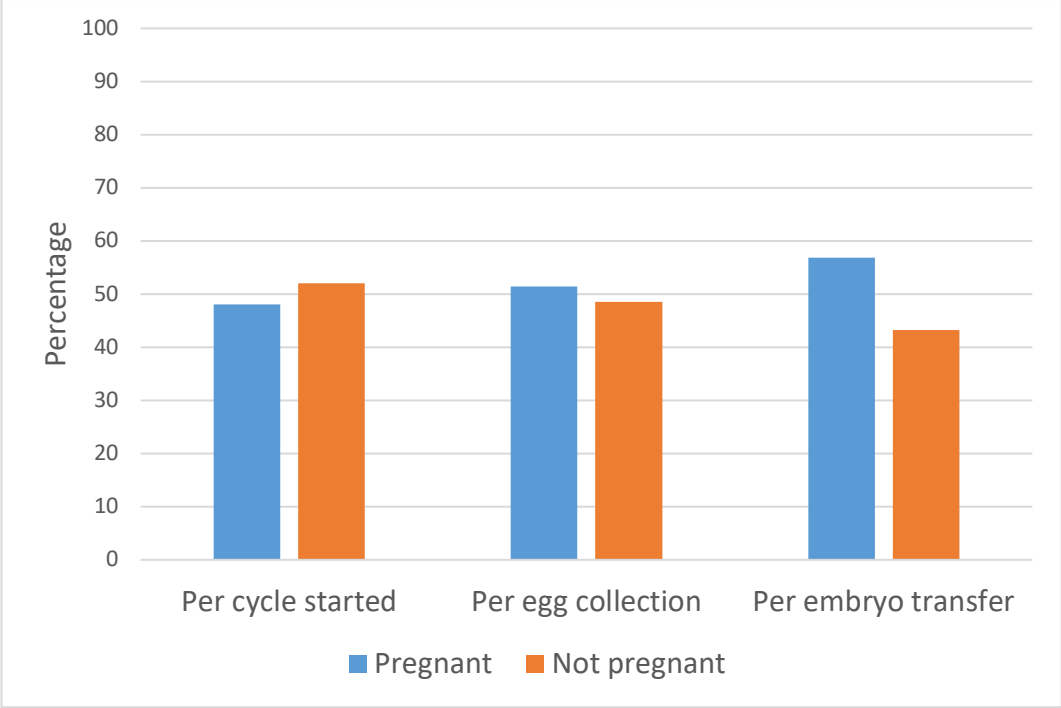

Figure 3.5: Positive urinary HCG test per IVF cycle.

3.8 Pregnancy outcome

Of those women who had a positive pregnancy test 10.9% (n = 12/110) had a biochemical pregnancy.

Ultrasound scans were performed at both 6 weeks and 8 weeks gestation. Seventy-five (76.5%; n = 75/98) were diagnosed with a singleton pregnancy on early pregnancy ultrasound and 23 (23.5%; n = 23/98) were diagnosed with a twin pregnancy. Twin pregnancy was confirmed by ultrasound scan at 6 weeks gestation, where two fetal heart pulsations were present and/or at 8 weeks, where two fetal heartbeats were present. There were no higher order multiple pregnancies. (Figure 3.6)

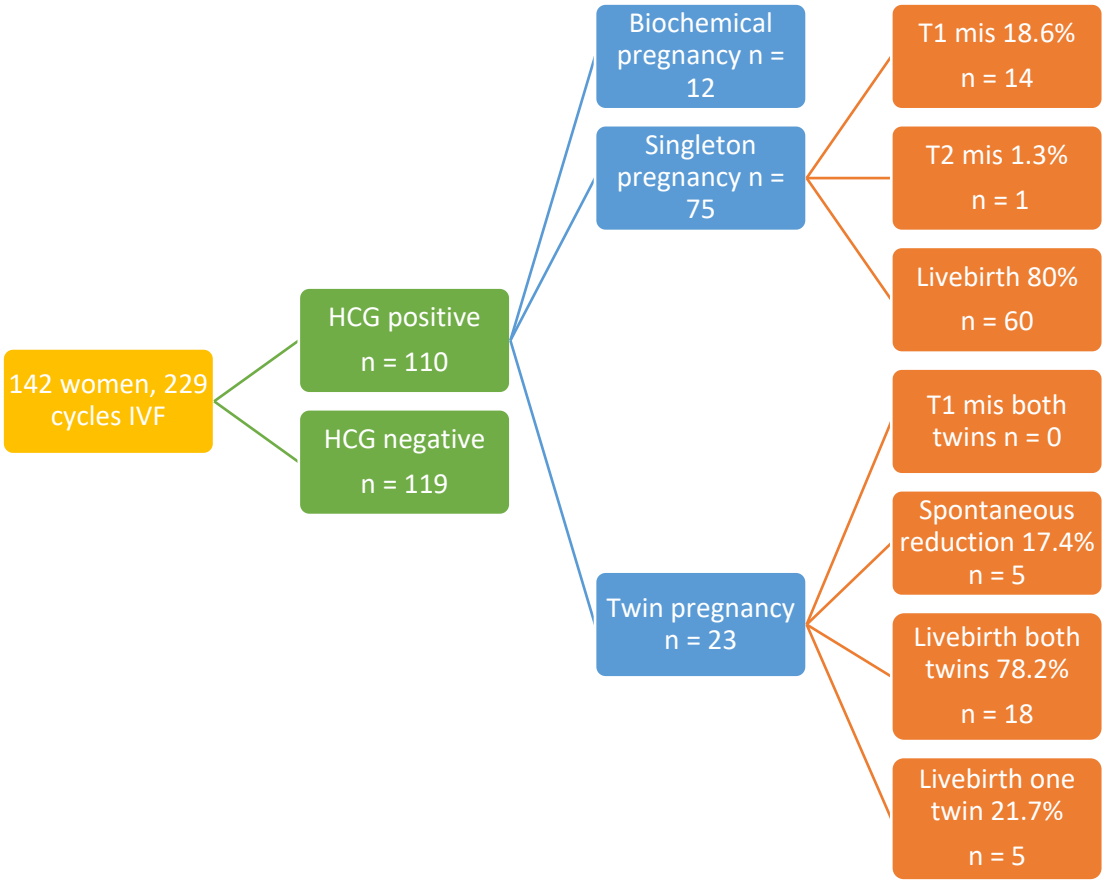

Figure 3.6: Summary of treatment cycle outcome according to pregnant or not pregnant T1 mis- first trimester miscarriage, T2 mis- second trimester miscarriage.

Of those women who became pregnant with twins, all had two embryos transferred, 51.8% were 37 years or older (n = 14/27). Only two of the women under 37 years had two good quality blastocysts in their first treatment cycle and therefore would have fulfilled the criteria for elective single embryo transfer (eSET).

The rate of first trimester miscarriage was 18.6% for singletons (n = 14/75). One singleton pregnancy resulted in a second trimester miscarriage. Four (17.4%; n = 4/23) twin pregnancies resulted in first trimester spontaneous reduction to singleton pregnancy (as confirmed at ultrasound at 8 weeks). One twin pregnancy resulted in a singleton livebirth following intrauterine demise of twin one at 26 weeks gestation. (Figure 3.7)

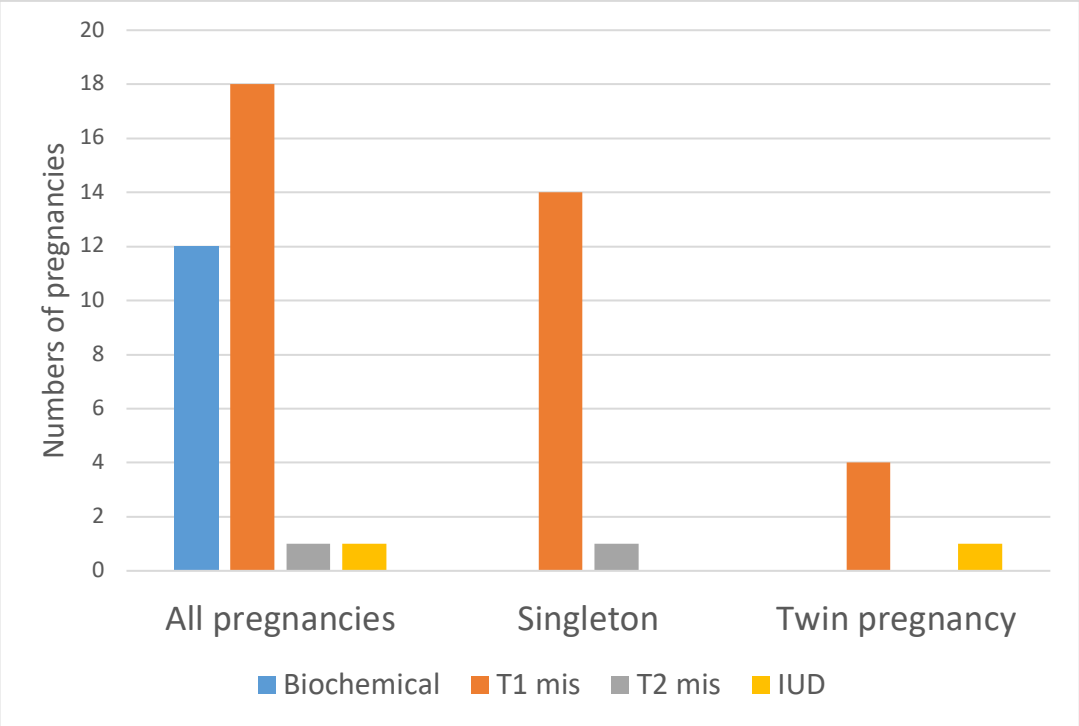

Figure 3.7: Pregnancy loss in all pregnancies and also according to number of fetuses. Note: T1 mis= First trimester miscarriage; T2 mis= Second trimester miscarriage; IUD= Intrauterine fetal demise

Over three-quarters of twin pregnancies (78.2%; n = 18/23) resulted in livebirth of both twins with 100% resulting in livebirth of one or both twins. Singleton pregnancies had a livebirth rate of 80% (n = 60/75) (Figure 3.8).

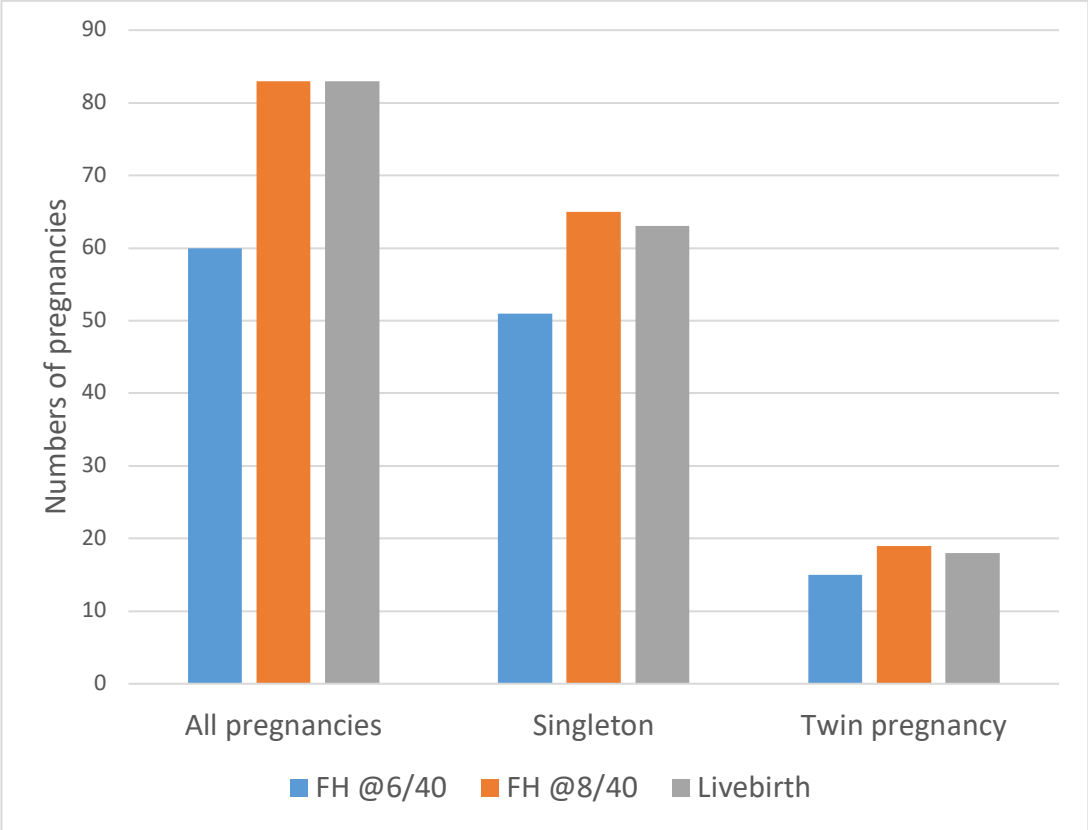

Figure 3. 8: Pregnancy outcome in all pregnancies and according to number of fetuses.

Note: FH = fetal heart pulsation visible on ultrasound scan.

3.9 Predicting chance of conception

3.9.1 Demographics and lifestyle

The cohort was next examined to assess if demographics and lifestyle predicted the chance of becoming pregnant from treatment or not as displayed in Table 3.5. None of the interrogated demographics or lifestyle factors (including smoking, alcohol intake and BMI) demonstrated a significant impact on the rate of pregnancy. Women who had previously conceived, but miscarried, from other assisted reproductive treatments i.e. intrauterine insemination (IUI) or ovulation induction (OII) were less likely to achieve pregnancy from IVF/ICSI, than women who had no previous pregnancy, however this finding did not reach significance.

Table 3.5: Odds Ratios for pregnancy/no pregnancy: demographics and lifestyle

	Pregnant *n* (%)	**Never pregnant *n* (%)**	**Model I OR (95% CI)**	**P - value**
Age				
Under 35 years	55 (52.9)	49 (47.1)	Ref	
Over 35 years	64 (51.2)	61 (48.8)	1.07 (0.64 - 1.80)	0.80
Ethnicity				
White Irish	104 (53.1)	92 (46.9)	Ref	
Any other	15 (45.5)	18 (54.5)	1.36 (0.65 - 2.84)	0.42
Education				
University	80 (54.4)	67 (45.6)	Ref	
Cert/Diploma	10 (55.6)	8 (44.4)	0.96 (0.36 - 2.56)	0.93
Secondary level	29 (45.3)	35 (54.7)	1.44 (0.80 - 2.60)	0.22
Previous SC[1] pregnancy				
No	108 (54.8)	92 (46.0)	Ref	
Yes	11 (37.9)	18 (62.1)	1.92 (0.86 - 4.28)	0.11
Previous ART pregnancy				
No	110 (54.2)	93 (45.8)	Ref	
Yes	8 (34.8)	15 (65.2)	2.218 (0.90 - 5.46)	0.08
Smoking				
No	105 (51.2)	100 (48.8)	Ref	
Yes	14 (58.3)	10 (41.7)	0.75 (0.32 - 1.77)	0.51
Alcohol				
No	25 (50.0)	25 (50.0)	Ref	
Yes	94 (52.5)	85 (47.5)	0.90 (0.48 - 1.69)	0.75
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	78 (53.4)	68 (46.6)	Ref	
Overweight and Obese (> 25.0)	41 (50.0)	41 (50.0)	1.15 (0.67-1.97)	0.62

Model I: Univariate unadjusted model.

1 SC= spontaneously conceived

3.9.2 Biochemistry

Biochemical markers were also assessed for their impact on the possibility of achieving pregnancy. The markers of ovarian reserve demonstrated significance for predicting the chance of becoming pregnant from treatment. Raised FSH (FSH > 11), which indicates reduced ovarian function, was associated with a significant reduction (60%) in the odds of achieving pregnancy (OR 0.4 (0.17 - 0.91); $p < 0.05$).

Women with an above average antral follicle count had a two-fold increase chance of pregnancy (OR 2.00; 95%CI 1.02 - 3.94) compared with those women with a low ovarian reserve, as evidenced by AFC < 7, also a significant result ($p < 0.05$). The final marker of ovarian reserve, AMH level, showed a trend to increased chance of pregnancy with a higher AMH (indicating a higher ovarian reserve) but this did not reach significance (OR 1.8 (0.94 - 3.43) $p = 0.08$) (Table 3.6).

3.9.3 Uterine dimensions

The volume of the uterus and the length of the uterus were assessed, according to their centile group (small uteri, average uteri and large uteri) and the occurrence of pregnancy or not. There was no impact of uterine dimensions, as measured by 2D and 3D ultrasound, on pregnancy rates from IVF/ICSI.

Table 3.6: Odd Ratios for pregnancy/no pregnancy: ultrasound and biochemical markers

	Never Pregnant *n* (%)	**Pregnant *n* (%)**	**Model I OR (95% CI)**	**p-value**
Cycle				
1	75 (52.8)	67 (47.2)	Ref	
2	32 (48.5)	34 (51.5)	1.19 (0.66 -2.13)	0.56
3 - 5	12 (57.1)	9 (42.9)	0.84 (0.33 - 2.12)	0.71
FSH				
≤ 10mIU/ml	94 (49.2)	97 (50.8)	Ref	
≥ 11mIU/ml	22 (71.0)	9 (29.0)	0.40 (0.17 - 0.91)	0.03
AMH				
1 - 9pmol/L	46 (59.0)	32 (41.0)	Ref	
10 - 22 pmol/L	32 (44.4)	40 (55.6)	1.80 (0.94 - 3.43)	0.08
> 22 pmol/l	18 (45.0)	22 (55.0)	1.76 (0.81 - 3.80)	0.15
TSH				
≤ 2.5mU/L	90 (54.2)	76 (45.8)	Ref	
≥ 2.6mU/L	18 (37.5)	30 (62.5)	1.97 (1.02 - 3.82)	0.04
AFC				
≤ 7	34 (58.6)	24 (41.4)	Ref	
8 - 12	49 (58.3)	35 (41.7)	1.01 (0.51 - 1.99)	0.97
≥ 13	36 (41.4)	51 (58.6)	2.00 (1.02 - 3.94)	0.04
U/S Volume				
< 47cm^3	38 (54.3)	32 (45.7)	Ref	
47 - 60 cm^3	37 (54.4)	31 (45.6)	0.99 (0.51 - 1.94)	0.98
> 60 cm^3	34 (49.3)	35 (50.7)	1.22 (0.63 - 2.38)	0.55
Ext os to fundus				
< 7cm	40 (61.5)	25 (38.5)	Ref	
7 - 7.6cm	26 (47.3)	29 (52.7)	1.79 (0.86 - 3.70)	0.11
> 7.6cm	37 (54.4)	31 (45.6)	1.34 (0.67 - 2.68)	0.41

Model I: Univariate unadjusted model.

3.10 Pregnancy complications

Overall, the rate of individual pregnancy complications was low, with gestational diabetes the most frequently occurring complication followed by intrauterine growth restriction (IUGR) as demonstrated in Table 3.7. Less than one third of the cohort had a pregnancy complication, as outlined (29.8%; n = 25/84).

Table 3.7: Pregnancy complications

Pregnancy complication	**Yes**	**No**
Gestational Diabetes	7 (8.3)	77 (91.7)
Pregnancy induced Htn[1]	1 (1.2)	83 (98.8)
Pre-eclampsia	4 (4.8)	80 (95.2)
IUGR[2]	6 (7.1)	78 (92.9)
PPROM[3]	1 (1.2)	83 (98.8)
Oligohydramnios	1 (1.2)	83 (98.8)
Obstetric cholestasis	2 (2.4)	82 (97.6)
Placental abruption	1 (1.2)	83 (98.8)
Congenital abnormality	2 (2.4)	82 (97.6)
Any of the above	25 (29.8)	59 (70.2)

Results displayed as n (%)

1 - Hen - hypertension, 2 - IUGR - intrauterine growth restriction, 3 - PPROM - pre-term pre-labour rupture of membranes.

All ongoing pregnancies were analysed for the occurrence of obstetric complications. Obstetric complications occurred in almost one-third of ongoing pregnancies (29.7%; n = 25/84). As displayed in Table 3.8, neither the demographic nor lifestyle factors analysed displayed any significance for the occurrence of obstetric complications.

Table 3.8: Odd Ratios for complications: demographics and lifestyle

	No complications ***n* (%)**	**Complications** ***n* (%)**	**Model I OR (95% CI)**	**P-value**
Age				
Under 35 years	23 (79.3)	6 (20.7)	Ref	
Over 35 years	24 (68.6)	11 (31.4)	1.76 (0.56 - 5.53)	0.34
Ethnicity				
White Irish	41 (71.9)	16 (28.1)	ref	
Any other	6 (85.7)	1 (14.3)	0.43 (0.05 - 3.83)	0.48
Education				
University	28 (71.8)	11 (28.2)	Ref	
Cert/Diploma	4 (80.0)	1 (20.0)	0.64 (0.06 - 6.35)	0.7
Secondary level	15 (75.0)	5 (25.0)	0.85 (0.25 - 2.90)	0.79
Previous spontaneously conceived pregnancy				
No	39 (73.6)	14 (26.4)	Ref	
Yes	8 (72.7)	3 (27.3)	1.05 (0.24 - 4.50)	0.95
Previous ART pregnancy				
No	40 (71.4)	16 (28.6)	Ref	
Yes	6 (85.7)	1 (14.3)	0.42 (0.05 - 3.74)	0.43
Smoking				
No	44 (75.9)	14 (24.1)	Ref	
Yes	3 (50.0)	3 (50.0)	1.26 (0.30 - 5.27)	0.75
Alcohol				
No	10 (76.9)	3 (23.1)	Ref	
Yes	37 (72.5)	14 (27.5)	1.26 (0.30 - 5.27)	0.75
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	32 (78.0)	9 (22.0)	Ref	
Overweight and Obese (> 25.0)	15 (68.2)	7 (31.8)	1.66 (0.52 - 5.31)	0.39

Model I: Univariate unadjusted model.

As displayed in Table 3.9, neither the biochemistry nor the ultrasound measurements performed prior to FSH stimulation was predictive for the occurrence of obstetric complications.

Table 3.9: Odd Ratios for complications: ultrasound and biochemical markers

	No Complications ***n* (%)**	**Complications** ***n* (%)**	**Model I OR (95% CI)**	**P-value**
Cycle				
1	28 (75.7)	9 (24.3)	Ref	
2	16 (72.7)	6 (27.3)	1.17 (0.35 - 3.88)	0.80
3 - 5	3 (60.0)	5 (40.0)	2.07 (0.30 - 14 - 44)	0.46
FSH				
≤ 10mIU/ml	43 (75.4)	14 (24.6)	Ref	
≥ 11mIU/ml	2 (50.0)	2 (50.0)	3.07 (0.40 - 23.87)	0.28
AMH				
1 - 9pmol/L	14 (77.8)	4 (22.2)	Ref	
10 - 22 pmol/L	15 (71.4)	6 (28.6)	1.40 (0.33 - 6.03)	0.65
> 22 pmol/l	11 (78.6)	3 (21.4)	0.96 (0.18 - 5.19)	0.96
TSH				
≤ 2.5mU/L	34 (75.6)	11 (24.4)	Ref	
≥ 2.6 mud/L	12 (70.6)	5 (29.4)	1.29 (0.37 - 4.47)	0.69
AFC				
≤ 7	5 (55.6)	4 (44.4)	Ref	
8 - 12	16 (72.7)	6 (27.3)	0.47 (0.09 - 2.36)	0.36
≥ 13	26 (78.8)	7 (21.2)	0.34 (0.07 - 1.60)	0.17
U/S Volume				
< 47cm^3	13 (72.2)	5 (27.8)	Ref	
47 - 60 cm^3	12 (80.0)	3 (20.0)	0.65 (0.13 - 3.33)	0.61
> 60 cm^3	20 (80.0)	5 (20.0)	0.65 (0.16 - 2.70)	0.55
Ext os to fundus				
< 7cm	8 (66.7)	4 (33.3)	Ref	
7 - 7.6cm	14 (82.4)	3 (17.6)	0.43 (0.08 - 2.42)	0.34
> 7.6cm	16 (80.0)	4 (20.0)	0.50 (0.10 - 2.54)	0.40

Model I: Univariate unadjusted model.

3.11 Pregnancy loss versus livebirth

Those who conceived were investigated for any factors that would indicate the chance of a successful outcome of pregnancy, i.e. livebirth as opposed to a pregnancy loss. None of the factors assessed demonstrated significance in terms of predicting livebirth or pregnancy loss.

Women with a previous pregnancy from ART (but resulted in miscarriage, as all recruits were nulliparous according to inclusion criteria) showed a trend for higher chance of livebirth compared with those without, this did not reach significance however. All results are displayed in Table 3.10.

Table 3.10: Odd Ratios for livebirth and pregnancy loss: demographics and lifestyle

	Pregnancy loss *n* (%)	**Live birth *n* (%)**	**Model I OR (95% CI)**	**P-value**
Age				
Under 35 years	12 (24.5)	37 (75.5)	Ref	
Over 35 years	15 (24.6)	46 (75.4)	0.99 (0.41 - 2.38)	0.99
Ethnicity				
White Irish	20 (21.7)	72 (78.3)	Ref	
Any other	7 (38.9)	11 (61.1)	0.44 (0.15 - 1.27)	0.13
Education				
University	15 (22.4)	52 (77.6)	Ref	
Cert/Diploma	2 (25.0)	6 (75.0)	0.87 (0.16 - 4.74)	0.87
Secondary level	10 (28.6)	25 (71.4)	0.72 (0.28 - 1.83)	0.49
Previous spontaneously conceived pregnancy				
No	26 (28.3)	66 (71.7)	Ref	
Yes	1 (5.6)	17 (94.4)	1.92 (0.86 - 4.28)	0.11
Previous ART pregnancy				
No	22 (23.7)	71 (76.3)	Ref	
Yes	5 (33.3)	15 (66.7)	2.22 (0.90 - 5.46)	0.08
Smoking				
No	25 (25.0)	75 (75.0)	Ref	
Yes	2 (20.0)	8 (80.0)	1.33 (0.27 - 6.70)	0.73
Alcohol				
No	8 (32.0)	17 (68.0)	Ref	
Yes	19 (22.4)	66 (77.6)	0.90 (0.48 - 1.70)	0.75
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	17 (25.0)	51 (75.0)	Ref	
Overweight and Obese (> 25.0)	10 (24.4)	31 (75.6)	1.03 (0.42 - 2.54)	0.94

Model I: Univariate unadjusted model.

Biochemical and ultrasound markers of fertility were examined for the cohort of women who conceived. Women with a normal AFC (8 - 12) had a 4-fold increased chance of livebirth compared to women with a reduced AFC of less than 7 (OR 4.09 (95% CI 1.24 - 13.45); $p < 0.05$). Women with a high AFC (> 22) also had an almost 4 - fold higher chance of livebirth compared to those with reduced ovarian reserve (OR 3.47 (95% CI 1.20 - 10.00); $p < 0.05$). Neither the length nor the volume of the uterus demonstrated any significance in terms of the rate of pregnancy loss or livebirth. (Table 3.11).

Table 3.11: Odd Ratios for livebirth and pregnancy loss: Ultrasound and biochemical markers

	Pregnancy loss *n* (%)	**Live birth *n* (%)**	**Model I OR (95% CI)**	**P-value**
Cycle				
1	18 (26.9)	49 (73.1)	Ref	
2	8 (23.5)	26 (76.5)	1.19 (0.46 - 3.11)	0.71
3 - 5	1 (11.1)	8 (88.9)	2.94 (0.34 - 25.18)	0.32
FSH				
≤ 10mIU/ml	24 (24.7)	73 (75.3)	Ref	
≥ 11mIU/ml	3 (33.3)	6 (66.7)	0.66 (0.15 - 2.83)	0.57
AMH				
1 - 9pmol/L	10 (31.3)	22 (68.8)	Ref	
10 - 22 pool/L	8 (20.0)	32 (80.0)	1.81 (0.62 - 5.33)	0.28
> 22 pool/l	4 (18.2)	18 (81.8)	2.05 (0.55 - 7.63)	0.29
TSH				
≤ 2.5mU/L	18 (23.7)	58 (76.3)	Ref	
≥ 2.6 mud/L	8 (26.7)	22 (73.3)	0.85 (0.33 - 2.24)	0.74
AFC				
≤ 7	11 (45.8)	13 (54.2)	Ref	
8 - 12	6 (17.1)	29 (82.9)	4.09 (1.24 - 13.45)	0.02
≥ 13	10 (19.6)	41 (80.4)	3.47 (1.20 - 10.00)	0.02
U/S Volume				
< 47cm^3	9 (28.1)	23 (71.9)	Ref	
47 - 60 cm^3	9 (29.0)	22 (71.0)	1.80 (0.52 - 6.17)	0.35
> 60 cm^3	7 (20.0)	28 (80.0)	1.35 (0.42 - 4.33)	0.61
Ext so to fundus				
< 7cm	8 (32.0)	17 (68.0)	Ref	
7 - 7.6cm	6 (20.7)	23 (79.3)	0.96 (0.32 - 2.85)	0.94
> 7.6cm	8 (25.8)	23 (74.2)	1.57 (0.51 - 4.85)	0.44

Model I: Univariate unadjusted model.

3.12 Preterm labor

The number of pregnancies that delivered preterm (delivery at ≤ 37 weeks gestation) was 19.3% (n = 16/63) with the number of very preterm deliveries (< 32 weeks) 1.2% (n = 1). Twin pregnancies accounted for over two-thirds of pre-term deliveries (68.8%; n = 11/16).

As there was only a small number of twin pregnancies detailed analysis of pregnancy outcomes amongst twins was not possible.

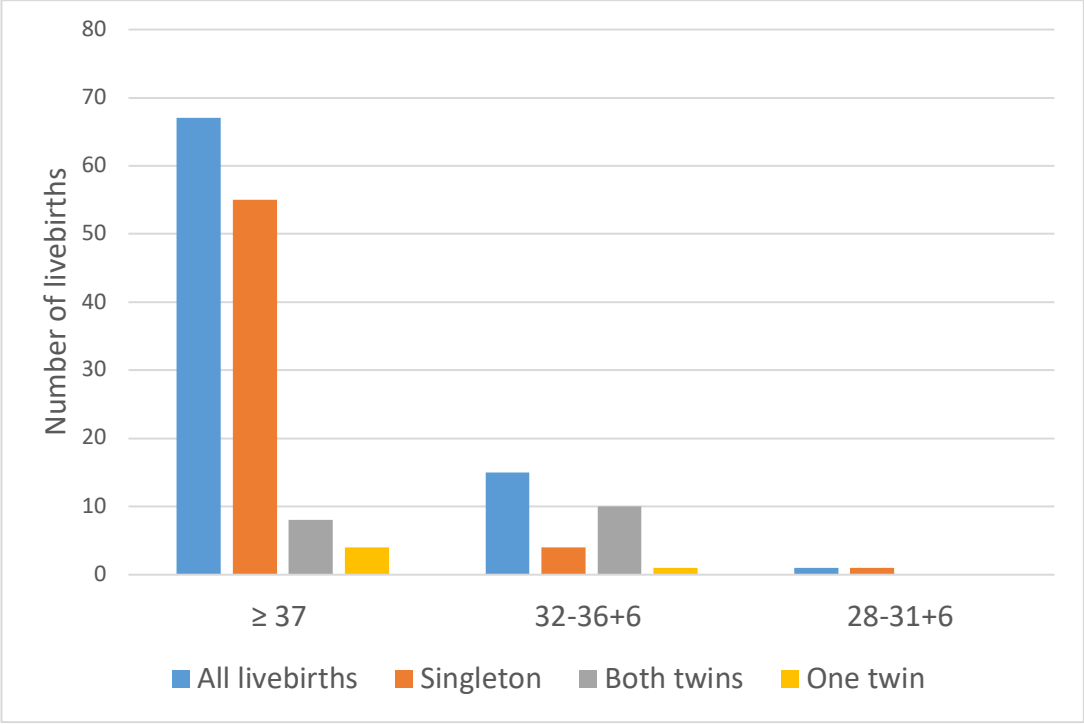

Figure 3.7: Gestation at delivery (weeks) according to all livebirths and also according to number of fetuses

There was no significant difference in the rate of pre-term delivery for women greater than 35 years old when compared to younger women. Demographics and lifestyle had no impact on the rate of pre-term delivery (Table 3.12).

Table 3.12: Odd Ratios for pre-term delivery: demographics and lifestyle

	Term ***n*** **(%)**	**Pre-term** ***n*** **(%)**	**Model I OR (95% CI)**	**P-value**
Age				
Under 35 years	30 (81.1)	7 (18.9)	Ref	
Over 35 years	37 (80.4)	9 (19.6)	1.04 (0.38 - 3.13)	0.94
Ethnicity				
White Irish	59 (81.9)	13 (18.1)	Ref	
Any other	8 (72.7)	3 (27.3)	1.70 (0.40 - 7.30)	0.47
Education				
University	42 (80.8)	10 (19.2)	Ref	
Cert/Diploma	4 (66.7)	2 (33.3)	2.10 (0.34 - 13.12)	0.43
Secondary level	21 (84.0)	4 (16.0)	0.80 (0.22 - 2.86)	0.73
Previous spontaneously conceived pregnancy				
No	55 (83.3)	11 (16.7)	Ref	
Yes	12 (70.6)	5 (29.4)	2.08 (0.61 - 7.11)	0.24
Previous ART pregnancy				
No	55 (77.5)	16 (22.5)	-	-
Yes	10 (100)	0 (0.0)	-	-
Smoking				
No	62 (82.7)	13 (17.3)	Ref	
Yes	5 (62.5)	3 (37.5)	2.02 (0.41 - 9.89)	0.39
Alcohol				
No	15 (88.2)	2 (11.8)	Ref	
Yes	52 (78.8)	14 (21.2)	2.02 (0.41 - 9.89)	0.39
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	38 (74.5)	13 (25.5)	Ref	
Overweight and Obese (> 25.0)	28 (90.3)	3 (9.7)	0.31 (0.08 - 1.20)	0.09

Model I: Univariate unadjusted model.

Similarly, biochemical and ultrasound markers of fertility were not predictive of pre-term delivery. Neither uterine dimensions nor uterine volume impacted on the rate of pre-term delivery with very similar distribution of pre-term deliveries in each measurement group (Table 3.13).

Table 3.13: Odd ratios for pre-term delivery: ultrasound and biochemical markers

	Term ***n*** **(%)**	**Pre-term** ***n*** **(%)**	**Model I OR (95% CI)**	**P-value**
Cycle				
1	40 (81.6)	9 (18.4)	Ref	
2	22 (84.6)	4 (15.4)	0.81 (0.22 - 2.93)	0.75
3 - 5	5 (62.5)	3 (37.5)	2.67 (0.54 - 13.56)	0.23
FSH				
≤ 10mIU/ml	59 (80.8)	14 (19.20)	Ref	
≥ 11mIU/ml	4 (66.7)	2 (33.3)	2.11 (0.35 - 12.70)	0.42
AMH				
1 - 9pmol/L	16 (72.7)	6 (27.3)	Ref	
10 - 22 pool/L	28 (87.5)	4 (12.5)	0.38 (0.09 - 1.56)	0.18
> 22 pool/l	12 (66.7)	6 (33.3)	1.33 (0.34 - 5.18)	0.68
TSH				
≤ 2.5mU/L	45 (77.6)	13 (22.4)	Ref	
≥ 2.6 mud/L	20 (90.9)	29 (9.1)	0.35 (0.07 - 1.70)	0.19
AFC				
≤ 7	9 (69.2)	4 (30.8)	Ref	
8 - 12	25 (86.2)	4 (13.8)	0.36 (0.07 - 1.75)	0.21
≥ 13	33 (80.5)	8 (19.5)	0.55 (0.13 - 2.23)	0.40
U/S Volume				
< 47cm^3	18 (78.3)	5 (21.7)	Ref	
47 - 60 cm^3	17 (77.3)	5 (22.7)	1.06 (0.26 - 4.32)	0.94
> 60 cm^3	23 (82.1)	5 (17.9)	0.78 (0.20 - 3.13)	0.73
Ext so to fundus				
< 7cm	14 (82.4)	3 (17.6)	Ref	
7 - 7.6cm	15 (65.2)	8 (34.8)	2.49 (0.55 - 11.31)	0.24
> 7.6cm	19 (82.6)	4 (17.4)	0.98 (0.19 - 5.11)	0.98

Model I: Univariate unadjusted model.

3.13 Mode of delivery

Almost half of the women had a vaginal delivery (48.2%; n = 40/83) and one quarter had a planned caesarean section (26.5%; n = 22/83). The remainder were delivered by emergency caesarean section 25.3% (n = 21/83) with two thirds occurring intrapartum (n = 14).

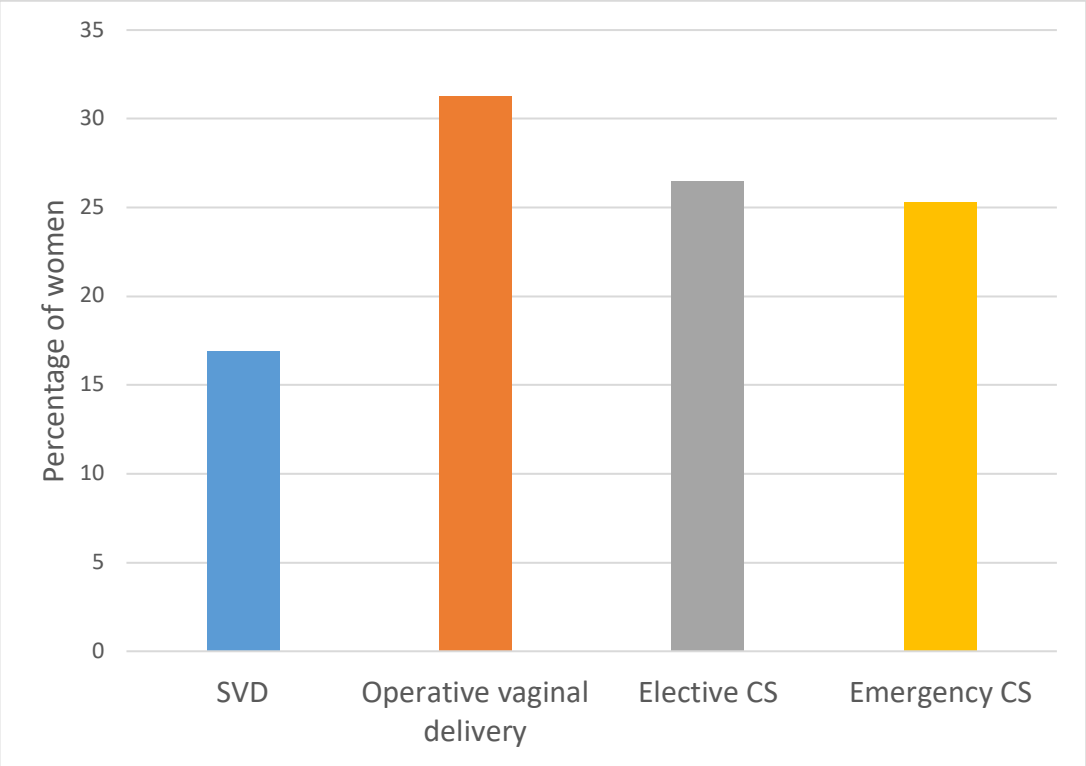

Figure 3.8: Mode of delivery

Note: SVD= spontaneous vaginal delivery; CS= Caesarean section

The population was analysed to assess risk factors for caesarean delivery None of the demographic and lifestyle factors, including age and BMI impacted on the mode of delivery (see Table 3.14).

Furthermore, neither fertility markers nor uterine dimensions including uterine volume helped determine risk of caesarean delivery versus vaginal delivery (Table 3.15).

Table 3.14: Odd Ratios for mode of delivery: demographics and lifestyle

	Vaginal ***n*** **(%)**	**CS** ***n*** **(%)**	**Model I OR (95% CI)**	**P-value**
Age				
Under 35 years	18 (48.6)	19 (51.4)	Ref	
Over 35 years	21 (46.7)	24 (53.3)	1.08 (0.45 - 2.59)	0.86
Ethnicity				
White Irish	32 (45.1)	39 (54.9)	Ref	
Any other	7 (63.4)	4 (36.4)	0.47 (0.13 - 1.75)	0.26
Education				
University	22 (43.1)	29 (56.9)	Ref	
Cert/Diploma	4 (66.7)	2 (33.3)	0.38 (0.06 - 2.26)	0.29
Secondary level	13 (52.0)	12 (48.0)	0.70 (0.27 - 1.83)	0.47
Previous spontaneously conceived pregnancy				
No	22 (48.5)	34 (51.5)	Ref	
Yes	7 (43.8)	9 (56.3)	1.21 (0.40 - 3.63)	0.73
Previous ART pregnancy				
No	32 (45.7)	38 (54.3)	Ref	
Yes	7 (70.0)	3 (30.0)	0.36 (0.09 - 1.51)	0.16
Smoking				
No	34 (45.9)	40 (54.1)	Ref	
Yes	5 (62.5)	3 (37.5)	1.13 (0.38 - 3.37)	0.83
Alcohol				
No	8 (50.0)	8 (50.0)	Ref	
Yes	31 (47.0)	35 (53.0)	1.13 (0.38 - 3.37)	0.83
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	27 (54.0)	23 (46.0)	Ref	
Overweight and Obese (> 25.0)	12 (38.7)	19 (61.3)	1.86 (0.75 - 4.63)	0.18

Model I: Univariate unadjusted model.

Table 3.15: Odd ratios for mode of delivery: Ultrasound and biochemical markers

	Vaginal *n* **(%)**	**CS** *n* **(%)**	**Model I OR (95% CI)**	**P-value**
Cycle				
1	23 (47.9)	25 (52.1)	Ref	
2	12 (46.2)	14 (53.8)	1.07 (0.41 - 2.79)	0.89
3 - 5	4 (50.0)	4 (50.0)	0.92 (0.21 - 4.11)	0.91
FSH				
≤ 10mIU/ml	33 (45.8)	39 (54.2)	Ref	
≥ 11mIU/ml	4 (66.7)	2 (33.3)	0.42 (0.07 - 2.46)	0.34
AMH				
1 - 9pmol/L	9 (40.9)	13 (59.1)	Ref	
10 - 22 pool/L	15 (48.4)	16 (51.6)	0.74 (0.25 - 2.23)	0.59
> 22 pool/l	10 (55.6)	8 (44.4)	0.55 (0.16 - 1.95)	0.55
TSH				
≤ 2.5mU/L	28 (49.1)	29 (50.9)	Ref	
≥ 2.6 mud/L	10 (45.5)	12 (54.5)	1.16 (0.43 - 3.10)	0.77
AFC				
≤ 7	4 (30.8)	9 (69.2)	Ref	
8 - 12	16 (57.1)	12 (42.9)	0.33 (0.08 - 1.35)	0.12
≥ 13	19 (46.3)	22 (53.7)	0.52 (0.14 - 1.94)	0.33
U/S Volume				
< 47cm^3	12 (52.2)	11 (47.8)	Ref	
47 - 60 cm^3	9 (42.9)	12 (57.1)	1.46 (0.44 - 4.78)	0.54
> 60 cm^3	12 (42.9)	16 (57.1)	1.46 (0.48 - 4.41)	0.51
Ext so to fundus				
< 7cm	9 (52.9)	8 (47.1)	Ref	
7 - 7.6cm	12 (54.5)	10 (45.5)	0.94 (0.26 - 3.34)	0.92
> 7.6cm	8 (34.8)	15 (65.2)	2.11 (0.59 - 7.60)	0.25

Model I: Univariate unadjusted model.

3.14 NICU Admission

Overall, the rate of admission to the neonatal intensive care unit (NICU) was low with only 20.5% of infants (n = 17/83) requiring NICU admission.

Maternal age greater than 35 years had a higher rate (28.3%; n = 13/46), although not reaching statistical significance, of an infant requiring NICU admission when compared with women under 35 years (10.8%; n = 4/37; p = 0.06) (Table 3.16). There were no other risk factors for NICU admission identified (Table 3.17).

Table 3.16: Odd Ratios for NICU; demographics and lifestyle

	Not admitted n (%)	**Admitted n (%)**	**Model I OR (95% CI)**	**p - value**
Age				
Under 35 years	33 (89.2)	4 (10.8)	Ref	
Over 35 years	33 (71.7)	13 (28.3)	3.25 (0.96 - 11.01)	0.06
Ethnicity				
White Irish	55 (76.4)	17 (23.6)	-	-
Any other	11 (100)	0 (0.0)	-	-
Education				
University	40 (76.9)	12 (23.1)	Ref	
Cert/Diploma	5 (83.3)	1 (16.7)	0.67 (0.07 - 6.27)	0.72
Secondary level	21 (84.0)	4 (16.0)	0.64 (0.18 - 2.21)	0.48
Previous spontaneously conceived pregnancy				
No	52 (78.8)	14 (21.2)	Ref	
Yes	14 (82.4)	3 (17.6)	0.80 (0.20 - 3.16)	0.75
Previous ART pregnancy				
No	56 (78.9)	15 (21.1)	-	-
Yes	10 (100)	0 (0.0)	-	-
Smoking				
No	63 (84.0)	12 (16.0)	Ref	
Yes	3 (37.5)	5 (62.5)	5.12 (0.63 - 41.70)	0.13
Alcohol				
No	16 (94.1)	1 (5.9)	Ref	
Yes	50 (75.8)	16 (24.2)	5.12 (0.63 - 41.70)	0.13
BMI (kg/m^2)				
Underweight and healthy (< 24.9)	41 (80.4)	10 (19.6)	Ref	
Overweight and Obese (> 25.0)	25 (80.6)	6 (19.5)	0.98 (0.32 - 3.04)	0.98

Model I: Univariate unadjusted model.

Table 3.17: Odd Ratios for NICU: biochemistry and ultrasound

	Not Admitted *n* (%)	**Admitted *n* (%)**	**Model I OR (95% CI)**	**p-value**
Cycle				
1	38 (77.6)	11 (22.4)	Ref	
2	22 (84.6)	4 (15.4)	0.63 (0.18 - 2.21)	0.47
3 - 5	6 (75.0)	2 (25.0)	1.15 (0.20 - 6.53)	0.87
FSH				
≤ 10mIU/ml	58 (79.5)	15 (20.5)	Ref	
≥ 11mIU/ml	4 (66.7)	2 (33.3)	1.93 (0.32 - 11.6)	0.47
AMH				
1 - 9pmol/L	17 (77.3)	5 (21.9)	Ref	
10 - 22pmol/L	25 (78.1)	7 (21.9)	0.95 (0.26 - 3.50)	0.94
> 22 pmol/L	15 (83.3)	3 (16.7)	0.68 (0.14 - 3.34)	0.64
TSH				
≤ 2.5mU/L	43 (74.1)	15 (25.9)	Ref	
≥ 2.6 mud/L	20 (90.9)	2 (9.1)	0.29 (0.06 - 1.38)	0.12
AFC				
≤ 7	9 (69.2)	4 (30.8)	Ref	
8 - 12	24 (82.8)	5 (17.2)	0.47 (0.10 - 2.15)	0.33
≥ 13	33 (80.5)	8 (19.5)	0.55 (0.13 - 2.23)	0.40
U/S Volume				
< 47cm^3	19 (82.6)	4 (17.4)	Ref	
47 - 60 cm^3	17 (77.3)	5 (22.7)	1.40 (0.32 - 6.07)	0.66
> 60 cm^3	23 (82.1)	5 (17.9)	1.03 (0.24 - 4.40)	0.97
Ext os to fundus				
< 7cm	15 (88.2)	2 (11.8)	Ref	
7 - 7.6cm	15 (65.2)	8 (34.8)	4.00 (0.73 - 22.05)	0.11
> 7.6cm	20 (87.0)	3 (13.0)	1.13 (0.17 - 7.60)	0.90

Model I: Univariate unadjusted model.

3.15 Summary

There was a high rate of livebirth amongst the cohort. All twin pregnancies resulted in the livebirth of at least one infant (i.e. there was no pregnancy loss that involved both twins). Lifestyle and demographics did not significantly impact on ART or pregnancy outcome. Uterine dimensions had no impact on ART or pregnancy outcomes. Markers of ovarian reserve i.e. AMH, FSH and AFC helped determine ART outcome and livebirth rate. There was a low rate of twin pregnancy amongst the women in the cohort, in spite of almost three quarters of all embryo transfers being double embryo transfers. This was identified in the early stages of the study. Furthermore, in the retrospective study, twin pregnancies had been identified as having more reasonable perinatal outcomes than was originally hypothesised, including a very low rate (1.5%) of extreme prematurity, therefore the decision was made to also follow the outcome of singleton pregnancies. The cross-sectional study (outlined in Chapter 4) was developed to assess other factors, including stress, that may impact on ART and pregnancy outcome.

Chapter 4 - The Cross-Sectional Study

Chapter 4 - The Cross-sectional Study - A survey-based prospective study of women attending for fertility consultation or treatment at Cork Fertility Centre

4.1 Methods

A patient survey was distributed to all women who were recruited to the prospective cohort and to a randomly selected women attending Cork Fertility Centre for consultation or treatment.

4.1.1 Background (Women's Health Cohort study, WHC)

The survey is a modified version of the validated survey used for the Women's Health Cohort study at Cork University Maternity Hospital (CUMH) (217). The WHC study explored the following potential risk factors for miscarriage among a population of pregnant women attending the early pregnancy assessment unit (EPU) of CUMH. The women attended EPU for either a first trimester scan or for their routine booking scan at 12 weeks gestation. The EPU provides ultrasound scans to women who present with pain and/or bleeding during the first trimester of pregnancy. It also provides ultrasound scans to asymptomatic women who have a history of miscarriage. All women are offered a booking scan at 12 weeks gestation for dating purposes. The WHC study assessed the following areas; socioeconomic characteristics, past reproductive history, diet and lifestyle factors, physical activity and partners' characteristics. The WHC survey was developed through a review of

the literature. For example, demographic questions reflected those collected by other national collections or census by the Central Statistics Office (CSO). Questions about diet and lifestyle factors were selected using the Pregnancy Risk Assessment Monitoring System (PRAMS), Ireland (218). The remaining sections were not collected using validated questionnaires. The WHC study survey was modified for use in this study by including questions directly related to subfertility and where questions referred to pregnancy, by modification to refer to 'trying to conceive'. The survey also included information on a range of traumatic events e.g. loss of job, separation or divorce, serious accident or illness, death of someone close, previous miscarriage, stillbirth and death of a child, based on a list developed by Maconochie et al. (138). Women were asked to indicate if they experienced a traumatic event in the last 12 months, more than 12 months ago, or not at all. The WHC study was approved by the institutional Clinical Research Ethics Committee (Reference: ECM 4 (iii) 10/01/12).

4.1.2 Survey details

The participants were invited to complete a paper based or web-based survey. The survey captured comprehensive demographic and lifestyle data relevant to the period of time they had been trying to conceive (for sample of survey see appendix viii) This included:

- Ethnicity, marital status, living arrangements, education and employment status
- Physical exercise- frequency and duration

- Dietary factors including caffeine intake, fruit and vegetable intake
- Smoking and alcohol
- Medications and illicit drug use
- Medical conditions
- Previous fertility and obstetric history where applicable
- A detailed survey on emotional/psychological wellbeing to measure specifically
    - Perceived stress - 10-item perceived stress scale (PSS-10) was developed as part of the women's health study to assess perceived stress. Using a 5-point scale ranging from never to very often, women were asked to indicate how often life situations were perceived as uncontrollable, unpredictable and stressful in the past month. Higher summary scores indicate greater perceived stress.
    - Emotional wellbeing - measured using a 6-item subscale of the RAND-36 (219). Women were asked to report how often they had felt happy, tired, worn out, nervous, downhearted or sad over the past four weeks. Responses were measured on a 6-point scale ranging from 'none of the time' (score of zero) to 'all of the time' (score of five). Higher summary scores indicate a more favourable state of emotional wellbeing.
    - Social support - measured using the Maternity Social Support Scale (220). This 6-item scale measures support from the woman's spouse, family and their wider social network on a 5-point scale ranging from 'never' (score of 1) to always (score of 5). Higher summary scores are indicating higher levels of social support.

- Outlook - measured using the Revised Life Orientation Test (LOT-R) which is a measure of dispositional optimism (221). This 10-item scale assesses individual differences in generalised optimism versus pessimism. Women chose from a 5-point scale ranging from 'I strongly disagree' (score of 1) to 'I strongly agree' (score of 5). Higher summary scores are indicative of an optimistic rather than a pessimistic outlook

4.1.3 Distribution of survey (web-based, paper based)

The paper-based survey was distributed at random to women attending the Cork Fertility Centre for consultation or treatment. The women completed the survey and returned it in a sealed envelope to the reception desk at the clinic. Three hundred fifty-six women in this cohort were invited to complete the survey and 208 women responded, resulting in a response rate of 58.4%. Women separately recruited for the prospective cohort study were invited by email, following completion of the initial assessment and signing of the consent form, to complete a web-based version of the survey using the online survey software site Survey Gizmo® (Widgix LLC, Boulder Colorado, U.S.A). One hundred twelve women in this cohort completed the survey resulting in a response rate of 78.9% (n = 112/142). The combined overall response rate was 64% (n = 320/498).

4.1.4 Data collection

The paper-based surveys were returned by the patient to Cork Fertility Centre once complete. Each woman was assigned a study identification number so that the results of the survey could be transcribed into and anonymised in an encrypted Microsoft Excel database. The outcome of treatment for those who proceeded for ART treatment was followed up through the electronic patient database, IDEAS V. 5.3 ™, Mellowood Medical. The results were then transcribed into an Excel dataset for further analysis.

For the women recruited for the detailed prospective study who completed the web-based survey, the online software collated the results which were then exported to a Microsoft Excel dataset, where the data was anonymised according to the patient's unique study identification number. Outcomes were followed prospectively through the IDEAS electronic patient database.

4.1.5 Outcome measurements

The specific outcomes measured were the occurrence of pregnancy (defined as positive urinary HCG test), first trimester miscarriage (defined as loss of an intrauterine pregnancy at ≤ 12+6 weeks gestation), second trimester miscarriage (defined as loss of an intrauterine pregnancy at 13 - 23+6 weeks gestation), intrauterine fetal demise (defined as a fetus with no signs of life in-utero after 24 weeks gestation), neonatal death (NND; defined as death in the first 28 days of life) and livebirth (liveborn infant weighting ≥ 500g and/or delivered at ≥ 24 weeks

gestation). The occurrence of pregnancy and miscarriage was collected prospectively at the fertility clinic as part of the routine follow up of IVF/ICSI outcome. Livebirth was recorded by fertility nurses either by the woman self-reporting i.e. by the woman contacting the clinic or by directly contacting the women postnatally. The survey results of those women who became pregnant were also compared to women who conceived spontaneously, recruited separately as part of the Women's Health Cohort Study (WHC) (222).

4.2 Introduction

The second study population was a questionnaire-based study of women attending the fertility clinic for consultation or treatment. In total, 320 surveys were completed and the results analysed according to outcome (see appendix viii for a sample of the survey). The specific outcome measures were the occurrence of pregnancy, miscarriage and livebirth. The survey results of those women who became pregnant were also compared to women who conceived spontaneously, recruited through the CUMH Women's Health Study (WHC).

4.3 Patient demographics - fertility clinic cohort

Three hundred and twenty (response rate 64%; n = 320/498) women responded to the survey with age range of 27 - 46 years with a mean age of 36 years ± 3.4 years. Two-thirds (64%; n = 199) were 35 years or older. Almost 90% (n = 282) were of white Irish ethnicity and almost 85% (n = 268) were married (Table 4.1).

From a lifestyle perspective, 60% (n = 183) were of normal BMI, with 38% (n = 116) being either overweight or obese. Only 5.8% (n = 18) were cigarette smokers.

When characteristics were compared to the women from the Women's Health Study (WHC) there was a greater proportion of women under 35 in the latter group (67.9%; n = 279 $p < 0.001$) and a higher number of single or co-habiting women in the WHC than those who attending the Cork Fertility Centre ($p < 0.001$) (Table 4.1).

The WHC had a lower number of women who had completed an undergraduate or postgraduate degree ($p < 0.001$) and were almost three times more likely to be smokers (15.8%; n = 63, $p < 0.001$). There was no significant difference in BMI between the two groups with approximately one third of both groups either overweight or obese (Table 4.1).

Table 4.1: Maternal characteristics of women who attended the fertility clinic compared to the women's health cohort who attended the Early Pregnancy Unit during the first trimester of pregnancy.

	Fertility Clinic n = 320	**WHC n = 411**	**p-value**
Age			< 0.001
Under 35 years	112 (36.0)	279 (67.9)	
35 years and older	199 (64.0)	132 (32.1)	
Ethnicity			0.002
White Irish	282 (89.3)	329 (78.9)	
Any other white background	25 (7.9)	64 (14.4)	
Other including mixed background	9 (2.8)	24 (5.8)	
Marital Status			< 0.001
Single	6 (1.9)	61 (14.6)	
Married	268 (84.5)	259 (62.1)	
Cohabitating	40 (12.6)	88 (21.2)	
Separated/Divorced/Widowed	3 (0.9)	8 (2.0)	
Education			< 0.001
Some Primary and/or secondary level	38 (12.0)	115 (17.3)	
Certificate and/or higher diploma	103 (32.5)	152 (36.5)	
Undergraduate or postgraduate degree	176 (55.5)	149 (35.9)	
Smoking status			< 0.001
Smoker	18 (5.8)	63 (15.8)	
BMI (kg/m^2)			0.545
Underweight (< 18.5)	6 (2.0)	7 (1.8)	
Healthy weight (18.5 - 24.9)	183 (60.0)	248 (65.1)	
Overweight (25.0 - 29.9)	72 (23.6)	82 (21.5)	
Obese (> 30.0)	44 (14.4)	44 (11.5)	

Data presented as n (%)

4.4 Life stressors - fertility clinic cohort versus WHC

Both groups replied to questions on stressors in the last 12 months. Both cohorts had high numbers reporting a stressful job. Reports of a stressful job were significantly more frequent amongst the fertility clinic cohort (50%; n = 160 v 40%; n = 167) ($p < 0.05$). The WHC women were more likely to report serious financial problems within the past 12 months ($p < 0.005$). The WHC had a higher proportion of women reporting a previous miscarriage (18.7% v 11.6%; $p = 0.005$) and a previous stillbirth (0/320 v 3/411 0.7%; $p = 0.05$) in the past 12 months. In both groups, one in 10 women reported the death of someone close in the previous year (Table 4.2).

Table 4.2: Reported stressful life events in the past 12 months by women attending the fertility clinic population and those in the women's health cohort.

	Fertility Clinic	**WHC**	**p - value**
Job generally demanding/stressful	160 (50.0)	167 (40.0)	0.028
Loss of job/job security	26 (8.1)	25 (6.0)	0.996
Separation/divorce	3 (0.9)	4 (1.0)	0.377
Serious financial problems	10 (3.1)	37 (8.9)	0.003
Accident	4 (1.3)	6 (1.4)	0.074
Serious illness	8 (2.5)	9 (2.2)	0.756
Serious illness of someone close	47 (14.7)	52 (12.5)	0.107
Death of someone close	32 (10.0)	46 (11.0)	0.001
Death of a child	0 (0.0)	1 (0.2)	0.152
Stillbirth	0 (0.0)	3 (0.7)	0.050
Previous miscarriage	37 (11.6)	78 (18.7)	0.005
Another stressful/traumatic event	21 (6.6)	27 (6.5)	0.874

Data presented as n (%)

4.5 Fertility clinic cohort - treatment outcomes

Of those women attending the fertility clinic, 290 (90%; n = 290/320) proceeded to treatment (ovulation induction, intrauterine insemination, IVF/ICSI) during the study period. Of those who underwent fertility treatment, 58.2% (n = 169/290) became pregnant.

As outlined in Table 4.3, a number of demographic and lifestyle factors were analysed for their impact on chance of conception. Age 35 years or older was the only patient characteristic that was near significance (p = 0.051) in determining

pregnant versus non-pregnant with women less than 35 years old being more likely to conceive.

Table 4.3: Patient characteristics of women who underwent fertility treatment according to the occurrence or not of pregnancy

	Pregnant	**Not Pregnant**	**p-value**
Age			0.051
Under 35 years	66 (39.1)	27 (27.3)	
35 years and older	103 (60.9)	72 (72.7)	
Ethnicity			0.637
White Irish	151 (90.4)	88 (90.7)	
Any other white background	12 (7.2)	7 (7.2)	
Other including mixed background	4 (2.4)	2 (2.0)	
Marital Status			0.499
Single	2 (1.2)	2 (2.0)	
Married	148 (87.6)	79 (80.6)	
Cohabitating	17 (10.1)	16 (16.3)	
Separated/Divorced/Widowed	2 (1.2)	1 (1.0)	
Education			0.162
Some Primary and/or secondary level	18 (10.7)	12 (12.2)	
Certificate and/or higher diploma	57 (33.9)	30 (30.6)	
Undergraduate or postgraduate degree	93 (55.2)	56 (57.2)	
Smoking status			0.292
Smoker	14 (8.7)	12 (12.7)	
BMI (kg/m^2)			0.250
Underweight (<18.5)	3 (1.8)	1 (1.1)	
Healthy weight (18.5 - 24.9)	95 (57.6)	63 (67.5)	
Overweight (25.0 - 29.9)	46 (27.9)	3 (17.2)	
Obese (> 30.0)	21 (12.7)	13 (14.0)	

Data presented as n (%)

4.6 Fertility clinic cohort - Impact of stress on conception

Analysis of individual life stressors in the preceding 12 months, including job stress, serious financial problems, or pregnancy loss did not reveal significance in terms of conceiving from fertility treatment. A small number of women had experienced serious illness in the preceding 12 months and demonstrated significance in terms of not achieving pregnancy (Table 4.4).

Table 4.4: Reported stressful life events amongst fertility clinic cohort.

	Pregnant	**Not pregnant**	**p-value**
Job generally demanding/stressful	83 (62.9)	56 (71.8)	0.187
Loss of job/job security	16 (16.8)	7 (21.9)	0.523
Separation/divorce	2 (2.4)	1 (4.0)	0.542
Serious financial problems	5 (5.6)	2 (7.1)	0.765
Accident	2 (2.3)	2 (7.4)	0.213
Serious illness	3 (3.5)	4 (13.8)	0.047
Serious illness of someone close	24.0 (24)	13 (13.3)	0.263
Death of someone close	17 (16.2)	10 (21.3)	0.448
Death of a child	-	-	-
Stillbirth	-	-	-
Previous miscarriage	21 (22.1)	10 (25.0)	0.715
Another stressful/traumatic event	12 (12.6)	7 (20.0)	0.291

Note: Categories are not mutually exclusive.
Data presented as n (%)

4.7 Fertility clinic cohort - Emotional wellbeing

Psychological factors were also analysed in terms of achieving pregnancy in those women who proceeded to fertility treatment. Overall, the group had medium to high rates of emotional wellbeing and this did not differ between those who conceived or failed to conceive from treatment. Rates of maternal social support were very high, 90.7% (n = 146) amongst those who conceived and 89% (n = 81) amongst those who failed to conceive. They had medium to low levels of perceived stress and average energy levels and no significance was noted in terms of achieving pregnancy (Table 4.5)

Table 4.5: Reported psychological factors amongst fertility clinic cohort.

	Pregnant	**Not pregnant**	**p-value**
Emotional Wellbeing			0.696
Low	44 (26.7)	27 (29.3)	
Medium	55 (33.3)	29 (31.5)	
High	66 (40.0)	36 (39.1)	
Maternal Social Support			0.954
Low	1 (0.6)	1 (1.1)	
Medium	14 (8.7)	9 (9.9)	
High	146 (90.7)	81 (89.0)	
Energy/Fatigue			0.299
Low	48 (29.4)	23 (24.7)	
Medium	68 (41.7)	41 (44.1)	
High	47 (28.8)	29 (31.2)	
Perceived Stress			0.393
Low	50 (30.3)	34 (36.2)	
Medium	66 (40.0)	39 (41.5)	
High	49 (29.7)	21 (22.3)	

Data presented as n (%)

4.8 Fertility clinic cohort - Pregnancy outcomes

The women who became pregnant from fertility treatment were further analysed according to livebirth and pregnancy loss. Pregnancy loss in this group included biochemical pregnancy and miscarriage. Almost half of the pregnant women (47.6%; n = 80) delivered a singleton and 23 (n = 13.7%) delivered twins.

Women 35 years or older were more likely to suffer a miscarriage (67.9%; n = 19) compared to women less than 35 years (32.1%; n = 9) (Table 4.6). There was no other patient characteristic that was significant in determining risk of pregnancy loss.

Table 4.6: Maternal characteristics of women pregnant following fertility treatment according to livebirth and pregnancy loss.

	Birth	**Pregnancy Loss**	**p-value**
Age			0.525
Under 35 years	56 (40.3)	9 (32.1)	
35 years and older	83 (59.7)	19 (67.9)	
Ethnicity			0.703
White Irish	124 (89.9)	25 (92.6)	
Any other white background	11 (8.0)	1 (3.7)	
Other including mixed background	3 (2.1)	1 (3.7)	
Marital Status			0.033
Single	2 (1.4)	0 (0.0)	
Married	124 (89.2)	23 (82.1)	
Cohabitating	13 (9.4)	3 (10.7)	
Separated/Divorced/Widowed	0 (0.0)	2 (7.2)	
Education			0.060
Some Primary and/or secondary level	13 (9.4)	5 (17.8)	
Certificate and/or higher diploma	48 (34.8)	8 (28.6)	
Undergraduate or postgraduate degree	77 (55.8)	15 (53.6)	
Smoking status			0.333
Smoker	3 (2.3)	1 (3.7)	
BMI (kg/m^2)			0.795
Underweight (<18.5)	2 (1.5)	1 (3.6)	
Healthy weight (18.5 - 24.9)	79 (58.5)	15 (53.6)	
Overweight (25.0 - 29.9)	36 (26.7)	9 (32.1)	
Obese (> 30.0)	18 (13.3)	3 (10.7)	

Data presented as n (%)

* Data are missing for one woman, for ethnicity

4.9 Fertility clinic cohort - Stress and pregnancy outcomes

The analysis of the responses to questions on individual life stressors revealed a higher rate of pregnancy loss amongst those who reported a stressful/demanding job ($p < 0.05$). A number of patients reported non-specified stressful life events and separation/divorce in higher numbers amongst the pregnancy loss group, reaching statistical significance, however the numbers are small thus limiting interpretation (Table 4.7).

Table 4.7: Reported stressful life events amongst women pregnant from fertility treatment in terms of livebirth and pregnancy loss.

	Birth	**Pregnancy Loss**	**p-value**
Job generally demanding/stressful	37 (33.3)	11 (57.9)	0.040
Loss of job/job security	13916.5)	3 (18.8)	0.823
Separation/divorce	0 (0)	2 (13.3)	0.002
Serious financial problems	3 (4.1)	1 (7.1)	0.611
Accident	1 (1.4)	1 (7.1)	0.191
Serious illness	3 (4.2)	0 (0.0)	0.454
Serious illness of someone close	19 (22.9)	5 (31.3)	0.475
Death of someone close	13 (14.8)	4 (25.0)	0.309
Death of a child	-	-	-
Stillbirth	-	-	-
Previous miscarriage	16 (20.3)	5 (31.3)	0.334
Another stressful/traumatic event	6 (7.8)	6 (35.3)	0.002

Note: Categories are not mutually exclusive.
Data presented as n (%)

4.10 Psychological factors and pregnancy outcomes

Psychological factors had no impact on the risk of pregnancy loss amongst the group. Those who achieved livebirth were less likely to have higher perceived stress. All women had high levels of maternal support (Table 4.8).

Table 4.8: Reported psychological factors amongst women pregnant from fertility treatment in terms of livebirth and pregnancy loss.

	Birth	**Pregnancy Loss**	
Emotional Wellbeing			0.413
Low	38 (28.1)	5 (17.9)	
Medium	43 (31.9)	12 (42.9)	
High	54 (40.0)	11 (39.3)	
Maternal Social Support			0.883
Low	1 (0.8)	0 (0.0)	
Medium	12 (9.0)	2 (7.7)	
High	120 (90.2)	24 (92.3)	
Energy/Fatigue			0.646
Low	39 (29.1)	8 (29.6)	
Medium	54 (40.3)	13 (48.1)	
High	41 (30.6)	6 (22.2)	
Perceived Stress			0.122
Low	44 (32.6)	6 (21.4)	
Medium	49 (36.3)	16 (57.1)	
High	42 (31.3)	6 (21.4)	

Data presented as n (%)

There were variations between the two groups in terms of demographics and lifestyle. However advanced maternal age (≥ 35 years) was the only characteristic that was near significance in terms of determining conception.

Stress did not demonstrate an impact on the chance of conceiving, apart from the small number who reported recent serious illness. The women generally reported high rates of emotional wellbeing.

Amongst the fertility cohort stress, particularly job-related stress, is associated with higher chance of pregnancy loss. Conversely, those who achieve livebirth are less likely to report high perceived stress.

4.11 Summary

There are some demographic and lifestyle differences between the fertility clinic cohort and the WHC, reflective of the populations studied. Amongst the fertility clinic cohort, overall, the women reported good emotional wellbeing and support. However, pregnancy loss was found to be higher amongst women with stressful or demanding jobs and those who reported non-specified stress and separation/divorce, although the numbers are small, thus limiting interpretation.

Discussion and Conclusion

Discussion

The demand for Assisted Reproductive Technologies (ART), particularly IVF/ICSI, is increasing, mainly due to the increasing age profile of women pursuing pregnancy for the first time (1, 223). The technology behind these treatments is evolving in the continued pursuit of higher live birth rates, in the large part, driven by commercial gain. In the face of increasing commercialism in ART and the drive for increasing success rates, it is important to maintain a focus on the aim of delivering a healthy mother and a term infant.

The aetiology of subfertility is quite heterogenous with both a male and female partner to consider and multiple individual factors (10). It is therefore often difficult to identify specific factors that may impact on conception rates and obstetric outcome.

The thesis originally focussed on predicting the outcome of multiple pregnancies conceived following IVF/ICSI. The original hypothesis was, due to the increased risk of preterm labour associated with multiple pregnancies, if a woman could be identified prior to IVF/ICSI treatment as being at high risk of preterm labour then she would be selected for an elective single embryo transfer. Conversely, if a woman was deemed low risk for preterm labour than it may be more reasonable to consider a double embryo transfer, allowing for a more individualised embryo transfer policy tailored to the risk profile of the woman.

The secondary aims were to identify pre-existing characteristics that may be associated with negative treatment outcome and adverse pregnancy outcome. This thesis was a broad assessment of multiple maternal characteristics including

physical, lifestyle, emotional/psychological, biochemical and ultrasound characteristics that may impact on IVF/ICSI success and subsequent pregnancy outcome.

The retrospective study, outlined on Chapter 2 found that both singletons and twins conceived through IVF/ICSI generally have good obstetric and perinatal outcomes. DCDA twins conceived by ART were more likely to be delivered at moderate pre-term gestations than spontaneously conceived twins. However, the rate of extreme pre-term delivery (< 28 weeks gestation), associated with the highest rate of neonatal morbidity and mortality, was in fact very low amongst both twins and singletons, including singletons originating from a twin pregnancy (VTS singletons). VTS singletons were found to have favourable outcomes with a high rate of term delivery and all infants liveborn. They were, however, more likely to be delivered by caesarean section than singletons.

The findings of the retrospective study were used to inform the direction of the thesis, in particular the prospective cohort study, which was then expanded to examine the outcomes of both singletons and multiples.

In the prospective cohort study of nulliparous women attending for IVF/ICSI treatment, 2D and 3D ultrasound was used to measure the dimensions of uterine length and 3D measurements of uterine volume. The study found no association between uterine dimensions, including uterine volume on conceptions rates, miscarriage rates and in particular on pre-term delivery rates. The study also examined other factors, including lifestyle and demographics and biochemical markers that may impact on treatment outcome or pregnancy outcomes. Not

surprisingly, women 35 years and older had a higher rate of miscarriage. The traditional markers of diminished ovarian reserve- raised FSH level and/or decreased antral follicle count was associated with lower chance of pregnancy and livebirth. Women with an average or high antral follicle count had a significantly higher rate of livebirth. The more recent introduction of AMH as a biochemical marker of ovarian reserve did not, however, show as robust an association with pregnancy rates and livebirth rates. Once pregnant, a history of diminished ovarian reserve did not confer excess obstetric risk.

Subclinical hypothyroidism at initial consultation was associated with a significantly higher rate of conception, most likely due to treatment with levothyroxine in order to maintain thyroid stimulating hormone (TSH) at < 2.5mU/l.

The thesis was further expanded with a focus on other factors that affect the wider ART population. The cross-sectional study assessed factors including psychological stress and emotional wellbeing that are more difficult to elucidate but may have an impact on ART and pregnancy outcome. It found that the women were well supported emotionally. Interestingly the study found that women who reported a stressful or demanding job in the last 12 months had higher rates of miscarriage as did a smaller number of women who reported other stressful life events or separation/divorce.

ART conceived pregnancy

Twin pregnancy is known to carry increased risks for both mother and fetuses. For the mother there is an increased risk of miscarriage, gestational hypertension and operative delivery. Maternal mortality is 2.5 times greater with a twin pregnancy (224). For the infants, most of the morbidity and mortality is associated with prematurity and lower birth weight. Approximately half of twins are born pre-term and 10% are born at less than 32 weeks gestation, compared with 1% of singletons. Perinatal mortality is 7 times higher for twins as compared with singletons and they have a ten-fold increased risk of admission to neonatal unit, with a six- fold increased rate of cerebral palsy. One in 12 multiple pregnancies end in death or disability of one or more babies (22, 224).

ART is known to result in increased rates of twin and higher order multiple (HOM) pregnancies. The rate of HOM pregnancies has dropped significantly, as has, to a lesser extent, the twin pregnancy rate, following guidance from several international regulatory committees advising clinics to increase their rates of elective single embryo transfer in order to avoid multiple pregnancy. In the UK the percentage of multiple pregnancies has dropped from just over 25% in 2008 to 11% in 2016, with many clinics achieving the target of less than 10%, as set by the Human Fertilisation and Embryology Authority's (HFEA) 'One child at a time' report 2006 (22, 224). In Ireland, in the absence of a national governing body and mandatory reporting of treatment cycle outcomes by fertility clinics, it is difficult to establish the true rate of multiple pregnancy arising from ART. Some, but not all, of the IVF clinics in Ireland

report to the European register compiled by ESHRE and these numbers indicate a decreasing rate for twin livebirths following IVF treatment (223).

The Retrospective study - A retrospective study of all twin and singleton pregnancies arising from IVF/ICSI treatment at Cork Fertility Centre

As part of this thesis, a detailed retrospective study was performed to assess the outcome of dichorionic diamniotic (DCDA) twin pregnancies conceived by ART (ART twins) over the period (2009 - 2012). DCDA twins were selected as they are the twins that result from two embryos being transferred and as such ART clinicians have control over the rate of DCDA twins resulting from ART - iatrogenic twinning. The aim was to assess the obstetric outcomes of IVF/ICSI conceived twin pregnancy in our population and to ascertain whether ART conceived twins had excess risks when compared to spontaneously conceived twins (5). As discussed above, twin pregnancy carries increased obstetric and perinatal risks but there are conflicting reports on whether assisted conception further increases these risks when compared to spontaneously conceived pregnancies with some studies reporting an increased risk of low birth weight, preterm birth (5 - 9) whereas other studies report no increased maternal or perinatal risk conferred by mode of conception. (10 - 13) One systematic review by Helmerhorst et al perinatal mortality in twins conceived using ART was 40% lower compared with spontaneously conceived twins (176).

Overall, the study found that twin pregnancies conceived through ART have similar outcomes to spontaneously conceived twin pregnancies. Twin pregnancy

itself resulted in a higher rate of pre-term delivery (51.3% spontaneously conceived v 49% ART, p = .879) irrespective of mode of conception. Twins conceived following ART were more likely to be delivered by caesarean section and had a higher rate of delivery at moderately preterm gestations (32 - 33+6 weeks) with an associated higher rate of RDS and neonatal hypoglycaemia. Importantly, in terms of severe neonatal morbidity, there was low (< 1.5%) and similar rates of extreme pre-term delivery (< 28 weeks gestation) amongst both ART and spontaneously conceived twins. All other perinatal outcome measures were comparable between the conception groups and there was no difference in perinatal mortality. Maternal outcomes were also similar with no increased risk of antenatal complications conferred by ART conception.

There is evidence that ART singletons have increased perinatal risks when compared to spontaneously conceived singletons, with reports of increased rates of pre-term delivery, low birth-weight and cerebral palsy even following adjustment for factors such as maternal age and parity (176, 199). The reasons for the increased adverse outcomes are poorly understood. Many studies have suggested that the underlying subfertility or indeed that the IVF/ICSI laboratory processes may be a risk factor with studies showing higher rates of adverse outcomes associated with increasing duration of involuntary childlessness (225).

Vanishing twin syndrome has been identified as a potential risk factor for obstetric complications, in particular pre-term delivery and low birthweight. Vanishing twin syndrome (VTS) affects between 10 - 30% of pregnancies following ART (206, 209, 226). VTS is the phenomenon whereby more than one gestational sac

is present on early ultrasound but due to loss or the second sac/fetus, the pregnancy continues as a singleton. Several studies have demonstrated an increased rate of adverse perinatal outcome for VTS singletons. A recent large study of 113,784 livebirths from fresh and frozen ART cycles in the UK, by Kamath et al 2018 demonstrated an increased risk of pre-term birth and low birthweight among VTS singletons when compared to singletons with only one gestational sac at early ultrasound (irrespective of whether single or double embryo transfer had occurred) (208).

The retrospective review was expanded from the original review of DCDA twins (2009 - 2012) to include all singletons and all DCDA twins conceived from ART at the fertility clinic (2002 - 2013). Less data was available on perinatal outcomes in these cohorts and no spontaneously conceived cohort of singletons or DCDA twins was available for comparison. The available data was limited to gestation at delivery, mode of delivery, birth weight and gender.

Almost three quarters of all pregnancies conceived during the time period studied were singleton, with 13.6% twin pregnancies and the remainder being biochemical pregnancies. The women who conceived a twin pregnancy were slightly older than those who conceived a singleton pregnancy. This may reflect the increased rate of double embryo transfer in older women. Elective single embryo transfer is practiced for women under 37 years of age with good quality blastocysts for selection. It may also reflect the likelihood that older women, due to diminishing oocyte numbers and quality, may require more than one IVF treatment cycle, and

the tendency to transfer more than one embryo if previous cycles have been unsuccessful.

Overall, the singleton pregnancy outcome was favourable. There was a high rate of livebirth (80.2%) with a vaginal delivery rate of 54.3% and a term delivery rate of 93.5%. Four neonatal deaths occurred. Not surprisingly, singletons were more likely to be delivered at term than twins. Twins were four times more likely to be delivered before 36 weeks gestation and have a higher rate of NNU admission. Twin pregnancy was more likely to result in miscarriage of one or both twins when compared with singleton pregnancy (34.4% v 19.4%).

Twin pregnancy outcomes however were favourable overall, with the majority of twins (88%) delivered at term or late pre-term gestation (34 - 36+6). One third of twins were delivered by vaginal delivery. Almost 9 in 10 twin pregnancies resulted in livebirth of one or both twins. Almost half (47.2%) of twin pregnancies resulted in livebirth of both twins. There was a high rate of vanishing twin syndrome (VTS), higher than the rates quoted in the literature and occurred most commonly due to first trimester miscarriage.

In our group of VTS singletons, all infants were liveborn with almost all delivered at term (93.1%). In contrast to many studies VTS singletons in the study group had similarly high rates of term delivery and birthweights as singletons. When compared with singletons, VTS singletons were more likely to be delivered by caesarean section (both emergency or elective, $p < 0.05$).

What is lacking from the retrospective review is the comparison of outcomes between the ART singletons and a similar group of spontaneously conceived singletons. This would aid in determining whether ART itself has an impact on singleton outcome.

The prospective cohort study - A prospective study of nulliparous women after attending for IVF/ICSI treatment at Cork Fertility Centre

One hundred and forty-two nulliparous women undergoing an IVF/ICSI cycle were recruited for the prospective study. The majority were undergoing IVF/ICSI due to diminished ovarian reserve. Three quarters of women had a positive pregnancy test, with ten percent resulting in a biochemical pregnancy. Of those women with ongoing pregnancies, approximately three quarters were singleton pregnancies and one-quarter were twin pregnancies.

Almost one-fifth (18.6%) of singleton pregnancies resulted in a first trimester miscarriage. As regards twin pregnancies, none of them resulted in a first trimester miscarriage of both twins. Four twin pregnancies had a spontaneous reduction of one twin (VTS) due to first trimester miscarriage and a further one resulted in VTS due to IUD at 26 weeks gestation, all resulting in a singleton livebirth.

Overall, the livebirth rate for both singletons and twins was high- all twin pregnancies resulted on a livebirth of at least one twin and 80% of singleton pregnancies resulted in a livebirth.

In terms of demographics, physical characteristics and lifestyle the group was very homogenous, largely owing to the fact that they were undergoing treatment at

a privately funded fertility clinic and were therefore required to self-fund their treatment as, in Ireland, there is no state-funded IVF/ICSI treatment. Reflecting increasing rates of subfertility with increasing age (related to diminishing ovarian reserve) the mean age of the women was 35.27 years. Interestingly, while the vast majority of women (90%) were non-smokers, over three quarters of women continued to drink alcohol while trying to conceive, the majority drinking more than 5 units of alcohol per week. However, neither cigarette smoking nor drinking alcohol was shown to impact on treatment or pregnancy outcomes.

Again, reflective of the population being studied, the majority (59.8%) had a lower antral follicle count and the most common aetiology was diminished ovarian reserve. Over 85% of women however, had a normal FSH level (≤ 10mIU/ml). Not surprisingly, those women with a raised FSH (≥ 11mIU/ml) had significantly reduced chances of achieving pregnancy (OR 0.4). In contrast, those women with an average antral follicle count (8 - 12 antral follicles) had a two-fold increased chance of achieving pregnancy.

There was a high rate (approximately 75%) of double embryo transfers over all cycles. The resultant twin pregnancy rate was 23.5% (n = 23). This twin pregnancy rate is higher than the HFEA 'One at a Time' aim of 10% (22). Cork Fertility Centre practices elective single embryo transfer (eSET) for women who are less than 37 years old with more than one good quality embryo available for transfer, this is in accordance with NICE guidance (18). In this instance, it is recommended that one embryo is selected for transfer and the remaining embryo (s) are cryopreserved for future use, with little impact on IVF success (when cumulative pregnancy rates from

the subsequent transfer of a frozen embryo as measured), and a reduction in iatrogenic twinning. Indeed, due to improving livebirth rates from cryopreserved embryos, there is increasing evidence to support eSET in women older than 37 years (3, 227). In the cohort of patients studied, only two women who had a double embryo transfer would have fulfilled the criteria for eSET therefore most double embryo transfers were appropriate.

The initial focus of the study was the assessment of the uterine cavity using 2D and 3D ultrasound to assess whether the volume of the uterus could predict the chance of preterm delivery, particularly in twin pregnancy. It was hypothesised that preterm delivery is associated with poorer neonatal outcomes and occurs more frequently in twin pregnancy, therefore, if a patient could be identified, in advance of her IVF/ICSI cycle, as being at lower risk of preterm delivery then she could be selected for double embryo transfer.

As the retrospective review of twin pregnancy outcomes progressed, it transpired that ART conceived twins had a higher rate of moderate pre-term delivery (32 - 33^{+6} weeks gestation) but overall pregnancy outcomes were still favourable. Out of the large cohort reviewed, the numbers of ART conceived twins, and indeed singletons, that delivered at extreme prematurity (< 28 weeks gestation) were very small, comprising only 9 twin infants (6 twins (1.9%; n = 6/316), 1 VTS singleton (0.8%; n = 1/133) and 2 singletons (0.1%; n = 2/1389)).

Furthermore, there are limitations of assessing the size of the uterine cavity in a non-pregnant woman. The identification of the internal cervical os on ultrasound of a non-gravid uterus proved difficult. Following the interrogation of the literature,

we identified one group who described how they identified the internal os 'The upper limit of the cervix was arbitrarily defined as a plane perpendicular to the cervical canal positioned at the inferior limit of the endometrial line' in their study on cervical length after LLETZ (215). During the literature review we identified one study by Fanchin et al (published oral abstract only) who reported on 79 women who underwent hysterosonometry (the measurement of the dimensions of the uterus from the external os to the fundus using 2D ultrasound techniques) (228). The group was divided according the 30th and 70th centile for size and the impact of the uterine dimensions was on the gestational age at delivery, severe pre-term delivery (< 32 weeks) and fetal mortality. All three outcomes were significantly more prevalent amongst women in the group with the smallest uteri (< 63mm).

Our population had both 2D ultrasound dimensions performed (external os to uterine fundus) and 3D ultrasound volume measurement of the uterus performed. The group were also divided according to the 30th and 70th centile for size and all outcome measures were studied according to this. There was no correlation found between uterine dimensions (both 2D and 3D) and any of the outcome measures. In particular, uterine dimensions had no impact on preterm delivery rates nor on any mode of delivery. The numbers of twin pregnancies, particularly those that delivered preterm, were too small to assess the impact on preterm delivery as a separate cohort.

When assessing predictors for conception from treatment neither lifestyle nor demographics were significant, which may be due to the homogeneity of the study group. Previous miscarriage from intrauterine insemination/ovulation

induction treatment was associated with lower pregnancy rates. Although not reaching statistical significance and small numbers of women, it may be reflective of diminishing egg quality in those women. Kupka et al found that one previous miscarriage had a positive effect on pregnancy rates but the reverse was seen with increasing numbers of prior miscarriage (229).

Interestingly, women in this study with subclinical hypothyroidism, a high TSH (> 2.5mU/l) but normal T4 at initial work up, had a two-fold increased rate of conception. The majority of those women were treated with levothyroxine with the aim of maintaining TSH levels at ≤ 2.5mU/l. There is evidence that suggests that women with subclinical hypothyroidism undergoing IVF/ICSI who are treated with levothyroxine have higher pregnancy rates. There is a higher rate of subclinical hypothyroidism in the subfertile population as compared to that of the general population (230). It is generally accepted that women planning pregnancy and pregnant women with subclinical hypothyroidism be treated with levothyroxine to reduce the risk of miscarriage in particular (231). There have been some studies that have also shown an improvement in pregnancy rates following levothyroxine treatment in women with subclinical hypothyroidism undergoing IVF/ICSI (232). More recent systematic reviews and meta-analyses show conflicting results as regards pregnancy rates but consensus on the reduction in the rate of miscarriage (233, 234).

Given that the cohort was, on average, of advanced maternal age (35 years or older), there was a low rate of obstetric complications amongst the group. Gestational diabetes was the more frequent complication and occurred more often

in women over 35 years of age, as might be expected but surprisingly there was no difference in BMI or history of PCOS in these women.

Once pregnant, a history of diminished ovarian reserve (high FSH, low AFC) was not predictive of obstetric complications. Markers for ovarian reserve, as would be expected, help predict the chance of pregnancy. Women with normal and high AFC had a two- and four- fold increased chance of livebirth respectively. Although the numbers are extremely small, thus limiting interpretation, there are similar findings in larger studies (235, 236). AMH did not show the same ability to predict pregnancy in our study. A meta-analysis by Tal et al assessed the utility of AMH in predicting IVF/ICSI outcome and demonstrated a weak association between AMH levels and pregnancy rates and livebirth rates with another review by Iliodromiti drawing similar conclusions (237, 238).

The cross-sectional study - A survey-based prospective study of women attending for fertility consultation or treatment at Cork Fertility Centre

There is much focus in the medical literature on the impact of lifestyle on the outcome of ART. Negative predictors of outcome include alcohol, smoking and obesity. Psychological and emotional stress has also been studied in ART patients. ART is known to be a stressful time for women, coping with their subfertility and the expectations of fertility treatments. There is no clear consensus in the medical literature on the impact of stress in IVF/ICSI outcome. This is most likely due to the heterogeneity of studies in terms of the type of stressor/stress/distress being

measured. The stress process is generally made up of three aspects: a stressor e.g. a life event, followed by the perception of stress, which then may lead to affective, behavioural and/or biological stress responses (i.e. distress) (239). Furthermore, there is much heterogeneity in the reporting of the outcome of assisted reproductive treatment. Many studies on ART outcome will report their findings according to the occurrence of a positive pregnancy test, some report the rate of 'clinical pregnancy' and some report the rate of livebirth. The definition for clinical pregnancy can vary from the presence of an intrauterine growth sac (irrespective of the presence of a fetal pole), to the presence of a fetal heart pulsation at 8 weeks gestation. This limits the interpretation of their results and the significance of what is deemed a successful outcome. The majority of studies show no clear association between stress and conception rates from ART including a large meta-analysis by Boivin et al which also determined there was no causal effect between pre-treatment stress in IVF/ICSI and treatment outcomes (134, 137). There are several studies however which have demonstrated an association with poorer outcome (133, 136). There is a more robust association between psychological stress and an increased rate of miscarriage, irrespective of mode of conception (7, 240).

In order to identify determinants of successful ART treatment women were invited to complete a comprehensive, validated questionnaire to collect data on demographics, lifestyle and emotional factors that may impact on the outcome of the treatment cycle or obstetric outcome.

The subfertility group, by the very fact of attending a privately-funded fertility clinic, was quite homogenous in terms of ethnicity, socio economic group and level

of education. No state-funded IVF clinic exists in Ireland that would allow the inclusion of a more heterogenous patient group. This group was compared to the Women's Health Cohort (WHC) which comprised women attending the Early Pregnancy Unit (EPU) in a state funded tertiary maternity hospital. Therefore, there were differences between the two populations in terms of smoking status, level of education and marriage rates. The subfertility group were older which may be implicated in the aetiology of their subfertility. The WHC were more likely to have a history of pregnancy loss. The EPU provides reassurance scans to women who have a history of pregnancy loss so this most likely explains this finding, rather than it being representative of women with no subfertility issues.

The cohort of subfertile women were surveyed on varying stressors and the impact on conception and miscarriage rates. Overall, the women were well supported and had good rates of emotional wellbeing. As regards the impact of stressors on conception rates, the small number of women who reported serious illness in the past 12 months were less likely to conceive. Once pregnant, women who reported stressful or demanding jobs, non-specified stressful life events and separation/divorce had a higher rate of pregnancy loss. This finding is supported by similar findings in the literature as discussed above. Women who achieved livebirth were less likely to have high rates of perceived stress. Unfortunately, the interpretation of this data is limited by the very small numbers but it suggests that stressors do not impact greatly on the chance of conception from ART but may be implicated in increased rates of miscarriage.

Strengths and limitations

There are several strengths to the study. These include: the prospective nature of the recruitment and follow up, including ultrasound images taken at the time of recruitment and in advance of commencing FSH stimulation. The retrospective review of both singleton and twin pregnancy and a more in-depth retrospective review of the twin pregnancy outcomes, compared with spontaneously conceived twin pregnancies helped inform the outcomes in the local population. It highlighted the positive outcomes of IVF/ICSI twin pregnancies.

The limitations have to be acknowledged. In the retrospective study, the review of singleton pregnancy outcomes was lacking complete data on parity and there was a relatively high number of missing data for gestation and mode of delivery. The cohort is also missing a spontaneously conceived control group for comparison.

The initial aim of the thesis was to assess the dimensions of the uterus with the hypothesis that smaller uteri would carry a higher risk of pre-term delivery, particularly in twin pregnancy. Therefore, by identifying those women with smaller uteri and avoiding iatrogenic twinning for them, a more selective eSET policy, tailored to risk, could be used, rather than a blanket eSET policy based on age and embryo quality. With the exception of one study on hysterosonometry by Fanchin et al, published only as an oral abstract, there was no prior studies on this area. Furthermore, it was only as the project evolved that we realised the difficulty of assessing the non-pregnant uterus due to the difficulty identifying the internal os, as discussed above.

The recruitment process was slow and it was specialised, therefore it was initially only one researcher who could perform a 3D ultrasound assessment of the uterus and there was only one ultrasound machine equipped with 3D technology that was only available on certain days. Furthermore, numbers recruited were not consistent across the study time period due to my leave of absence. Two fertility nurse specialists were trained to continue recruiting in my absence, however, due to the constraints of also running a busy ultrasound scanning list while recruiting, the number of women recruited in that period was low. These factors impacted on numbers recruited.

The unpredictability of cycle success (IVF treatment carries approximately a 38% livebirth rate at Cork Fertility Centre) led to a longer follow up time in order to allow the assessment of outcomes of further treatment cycles so that more pregnancies could be captured.

Initially it was hoped that the numbers of twin pregnancies would be much higher to allow for a separate analysis of twin pregnancy outcomes. The difficulty is the low rate of twin pregnancy resulting from IVF/ICSI and no ability to predict which patient will have a twin pregnancy, or indeed get pregnant, from treatment, in a clinic with almost 800 IVF/ICSI cycles per year. Therefore, in order to recruit the maximum number of twin pregnancies, it would have been necessary to recruit almost all nulliparous women coming through the clinic for treatment and due to the constraints described above, in particular the access to the 3D equipped ultrasound machine, this was not feasible.

Overall, a low number of pregnancies was the result with limitations on interpreting the data. In particular the number of twin pregnancies in the recruited cohort was extremely low, meaning that no meaningful separate analysis on twin pregnancy outcomes could be performed.

The study group was very homogenous and not reflective of the general population, owing to the fact that they were required to self-fund their fertility treatment. As there is no state-funded IVF/ICSI treatment available in Ireland, there was no option to expand the study to include a more diverse population.

Future Work

Reflecting on the methods, results and limitations of the study, I feel that there are several things that could have been done differently and several areas that warrant future work.

Certainly, a larger cohort of patients in the prospective cohort study of IVF/ICSI outcome may have yielded more significant findings. A larger cohort may have resulted in more twin pregnancies in the study, allowing for a separate analysis of twin outcomes, thus fulfilling the original objective of the study.

The twin outcomes as demonstrated in the retrospective study were reassuring however. Certainly, it is not to be disputed that a twin pregnancy (irrespective of mode of conception) carries excess obstetric risk as compared to a singleton pregnancy but it is reassuring that ART conception does not escalate this risk significantly further. There are some authors now suggesting that the original

risks attributed to IVF conceived twins have been over-inflated, and that, coupled with the increased adverse outcomes associated with IVF/ICSI conceived singleton pregnancy, a blanket eSET policy is in fact not warranted. (241) Perhaps the focus should not be on avoiding twins at all cost in IVF/ICSI with a blanket eSET policy based on age, but to avoid twins in the higher-risk woman e.g. advanced maternal age, medical co-morbidities, obesity, previous pre-term labour, older women using donor oocytes.

Thus, upon reflection, perhaps the thesis would be more representative of the population as a whole and more likely to capture the higher-risk woman, if it included multiparous women, women with pre-existing medical co-morbidities and women attending state-funded fertility treatment. Currently, there is no state-funded IVF in Ireland resulting in a large population of subfertile couples who cannot afford to self-fund IVF treatment. Ideally, future work would involve incorporating both private and state-funded IVF clinics in order to study a more heterogenous group of patients. The population in this study was very homogenous in terms of demographics i.e. almost all white Irish, well-educated and non-smokers.

The cross-sectional study found a link between stress peri-conceptually and first-trimester miscarriage and similar findings have been published in the literature. Unfortunately, the associations between particular stressors and lower conception rates or first trimester miscarriage could not be confidently interpreted as the number of women in the cohort was too small. A larger, prospective study may yield interesting results and is worthy of future work. There is certainly a role for further

studies on stress peri-conceptually and the outcome of pregnancy in terms of first trimester miscarriage.

Conclusion

The thesis as a whole has demonstrated favourable outcomes for both twin and singleton pregnancies conceived through IVF/ICSI. Interestingly, in spite of the frequently acknowledged stressors associated with undergoing ART, there was no indication of an impact on pregnancy rates. There was a suggestion, supported by the literature, that stress impacted on miscarriage rates however, which may warrant further investigation with larger numbers and consideration of stress management strategies. Uterine dimensions had no impact on the success of a treatment cycle nor on pregnancy outcome.

It can be concluded that twin pregnancy outcomes from IVF/ICSI are not dissimilar to those of spontaneously conceived twins. However, twin pregnancies, irrespective of mode of conception, are associated with higher rates of pre-term delivery and NICU admission.

During ART there is an element of control over the number of embryos being transferred and the resultant rate of twin pregnancy. Therefore, in the absence of any robust predictors of successful, or indeed adverse, outcome it is preferable to aim for single embryo transfer and a singleton pregnancy. Couples with two good quality embryos should be counselled regarding the increased risk of iatrogenic twinning and its implications, associated with double embryo transfer.

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Appendices

Appendix i - Clinical Research Ethics Committee Approval for thesis

UCC

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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our ref: ECM 4 (bbbbb) 07/05/13

26th April 2013

Dr Keelin O'Donoghue
Consultant Obstetrician & Gynaecologist
Cork University Maternity Hospital
Wilton
Cork

Re: Predicting successful outcome of multiple pregnancy after assisted reproductive technologies (ART).

Dear Dr O'Donoghue

Expedited approval is granted to carry out the above study at:

- Cork Fertility Centre
- Cork University Maternity Hospital.

The following documents were approved:

- Application Form
- Study Protocol
- Information Leaflet
- Consent Form Version 1 dated 18th April 2013
- Patient Weight Record
- Study Questionnaire.

We note that the co-investigators involved in this study will be:

- Dr Minna Geisler and Dr John Waterstone.

Yours sincerely

Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

Appendix ii - Clinical Research Ethics Committee Approval for amendment to Women's Health Study and Miscarriage

UCC
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University College Cork, Ireland

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our ref: ECM 3 (www) 07/05/13

26th April 2013

Dr Keelin O'Donoghue
Consultant Obstetrician & Gynaecologist
Cork University Maternity Hospital
Wilton
Cork

Re: Women's Health Study and Miscarriage

Dear Dr O'Donoghue

The Chairman approved the following:

- Amendment Application Form
- Addition of Dr Minna Geisler as co-investigator.

If the questionnaire to be used in this part of the research has not been approved previously please send it for approval prior to use.

Yours sincerely

Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

Appendix iii – Cork University Maternity Hospital Twins Database

Variable	Description	Variable	Values
PATIENT_TYPE	Public of Private Patient	Categorical	Public Private Unknown
MATERNAL_AGE	Maternal age in years at booking	Integer	
PARITY	Parity	Categorical	Nulliparous Multiparous
Conception	How this pregnancy was conceived	Categorical	Spontaneous Artificial reproduction
Mode of ART	If artificial reproduction technology was used, which mode was used	Categorical	IVF ICSI OII Donor egg Donor sperm FET IUI
Chronicity	Chronicity of the multiples	Categorical	MCMA MCDA DCDA Conjoined
PRENATAL_STEROIDS	Were prenatal steroids administered in this pregnancy	Categorical	Yes No Unknown
GESTDM	Gestational diabetes in this pregnancy	Categorical	Yes No Unknown
PREECLAMPSIAPIH	Was preeclampsia/PIH diagnosed in this pregnancy	Categorical	Yes No Unknown
OBSTETRIC_CHOLESTASIS	Was obstetric cholestatsis diagnosed in this pregnancy	Categorical	Yes No Unknown
T2 MISCARRIAGE	Did the pregnancy result in a second trimester miscarriage	Categorical	Yes No Unknown
IUD	Did the pregnancy result in an intrauterine death	Categorical	Yes No Unknown

PTD	Did the pregnancy result in pre-term delivery	Categorical	Yes No Unknown
PTD_ONSET	Onset of labour for pre-term delivery	Categorical	Spontaneous labour Induction of labour Elective CS Emergency CS No trial of labour
DELIVERY_GESTATION	Gestation of delivery	Categorical	< 28 28-32 32^{+1} – 33^{+6} 34-36^{+6} ≥ 37
MODE_OF_DELIVERY	Mode of delivery	Categorical	Spontaneous vaginal delivery Breech delivery Instrumental delivery Elective caesarean section Emergency caesarean section Emergency CS no trial of labour
GESTATION	Gestational age at delivery	Integer	Weeks 0-43 Day 0-7
SEX	Sex of the infant	Categorical	Male Female
WEIGHT	Birthweight in grams	Integer	
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration (Apgar) summary score at 1 minute and 5 minutes after birth	Integer	
OUTCOMES	Any adverse outcome during pregnancy/following birth	Categorical	IUGR TTTS Fetal anomaly Intrauterine death Prematurity

			Stillbirth Neonatal death NICU admission
NND_COMPLICATIONS	Neonatal complications following birth	Categorical	Haematological Photoxx Jaundice Retinopathy of prematurity Chronic lung disease Hypoxic-ischemic encephalopathy, Necrotizing enterocolitis Sepsis Respiratory distress syndrome

Appendix iv - Maternal and Infant characteristics IDEAS database codebook

Variable	Description	Variable	Values
Mothers name	Maternal forename and surname	String	Free text
Partner's name	Partner forename and surname	String	Free text (if applicable)
Due date	Estimated date of delivery	Date	DD-MM-YYYY
Date of Outcome	Date of delivery or diagnosis of pregnancy loss	Date	DD-MM-YYYY
Gestation (wk/days)	Gestation at date of outcome	Integer	Weeks 0-43 Day 0-7
Reason for delivery	Indication for delivery	String	Free text
Birth presentation	Presentation at delivery	Categorical	Vertex Breech Transverse Brow Face
Method of delivery	Mode of delivery	Categorical	Spontaneous vaginal delivery Instrumental delivery Elective caesarean section Emergency caesarean section
Outcome info source	Outcome of the latest pregnancy	Categorial	Ectopic pregnancy Late miscarriage Late neonatal death Live birth Lost to follow-up Molar Negative Stillbirth Therapeutic abortion
Reason for termination		String	Free text

Complications at birth	Any complications identified at birth	Categorical	Intrauterine Growth Restriction Insert a comment (free text)
Gestational age (wk/days)	Gestational age at delivery	Integer	Weeks 0-43 Day 0-7
Sex	Sex of the infant	Categorical	Male Female
Weight (g)	Birthweight in grams	Integer	
Apgar score (1&3 min)	Appearance, Pulse, Grimace, Activity, and Respiration (Apgar) summary score at 1 minute and 3 minutes after birth	Integer	
Birth trauma	Any maternal or infant trauma experienced during birth	String	Free text
Birth defect	Any birth defect diagnosed during pregnancy and/or following delivery	Categorical	Aicardi syndrome Down syndrome Edwards syndrome Encephalocele Haemangioma Imperforate anus Polycystic kidney Spina bifida Occulta Trisomy 21 Cardiac murmur Cardiac defect Cleft chin Cleft palate Genetic defect Limb defect Under investigation Insert a comment (free text)
Date of death	Date of death of liveborn infant	Date	DD-MM-YYYY

Appendix v - Maternal and Infant characteristics Cork Fertility Centre database codebook

Variable	Description	Variable	Values
Name	Maternal forename and surname	String	Free text
Due date	Estimated date of delivery	Date	DD-MM-YYYY
CFC*	Cork fertility centre medical record number	Numeric	Numeric
Single/Twin	Number of fetuses in this pregnancy	Integer	
Date of delivery	Date of delivery	Date	DD-MM-YYYY
Gestational age	Gestation at date of outcome in weeks and days	Integer	Weeks 0-43 Day 0-7
Delivery Method	Mode of delivery	Categorical	Spontaneous vaginal delivery Instrumental delivery Elective caesarean section Emergency caesarean section
Gestational age (wk/days)	Gestational age at delivery	Integer	Weeks 0-43 Day 0-7
Sex	Sex of the infant	Categorical	Male Female
Weight (g)	Birthweight in grams	Integer	

Appendix vi - Screen tests database codebook

Test	Date	Result	Remarks
AMH- Pre-Infertility Test	DD-MM-YYYY		Free text
FSH test	DD-MM-YYYY		Free text
Free T4	DD-MM-YYYY		Free text
LH test	DD-MM-YYYY		Free text
Oestrogen test	DD-MM-YYYY		Free text
Progesterone test	DD-MM-YYYY		Free text
Prolactin	DD-MM-YYYY		Free text
Rubella	DD-MM-YYYY		Free text
T4&TSH	DD-MM-YYYY		Free text
TSH	DD-MM-YYYY		Free text

Appendix vii - Sample Consent Form

CONSENT BY SUBJECT

FOR PARTICIPATION IN RESEARCH PROTOCOL

Section A

Protocol Number:_____ Patient Name:_____

Participant Study I.D: _____

Title of Protocol:
Predicting successful outcome of multiple pregnancy after assisted reproductive technologies (ART).

Doctors Directing Research: Dr. Keelin O'Donoghue, Dr. John Waterstone, Dr. Minna Geisler
Phone: (021) 4920500

You are being asked to participate in a research study. The doctors at University College Cork study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent. This consent form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

Section B

I. NATURE AND DURATION OF PROCEDURE(S):

This aim of this study is to identify factors indicating that, if a particular woman were to become pregnant with twins, she would be at increased risk of early (preterm) labour (labour less than 37 weeks of pregnancy) or other pregnancy complications (e.g. miscarriage, high blood pressure, diabetes of pregnancy, admission of the baby to the neonatal intensive care unit).
The study involves taking measurements of the womb and cervix using a 3D transvaginal scan (several similar scans are carried out routinely in the course of every IVF/ICSI cycle). During the scan, a thin tube (catheter) may be inserted into the womb (a similar procedure that occurs at embryo transfer) and a small amount (5mls-10mls; 1-2 teaspoonfuls) of sterile saline solution may be passed through the tube, to outline the cervix and womb cavity (saline sonohysterography). The scan will be performed at the Cork Fertility Centre by Dr. Minna Geisler.

You will be asked to complete a questionnaire and your weight and height will be recorded. Blood samples (approx. 20mls; 4 teaspoonfuls) will be taken (for Anti-Mullerian Hormone levels, HbA1C and Collagen types I, III and IV). The samples for collagen levels will be stored at Cork University Maternity Hospital for analysis later in this study.

In the event that you become pregnant with non-identical twins, Dr. Geisler will follow you throughout pregnancy at certain time points. Two further transvaginal scans will be performed at 18 and 22 weeks to check for changes in the cervix that are linked to preterm labour. You will be asked to record your weight each week.

Consent Form Version No. I – 18/04/2013

The following is standard management for all twin pregnancies and will be part of the study.
Ultrasound scans will be performed regularly (18, 24, 28, 32 and 36 weeks) to check the fluid around the babies and to check the weight of the babies. A glucose tolerance test (GTT- a blood test to check for diabetes of pregnancy) will be performed at 28 weeks.

II. POTENTIAL RISKS AND BENEFITS:

By participating in this study you will assist in improving our knowledge of preterm labour in twin pregnancies. The ability to identify women with risk factors for preterm labour, before they undergo embryo transfer, would allow fertility specialists to decide whether it is safe for a particular woman to have a twin pregnancy (in which case two embryos would be transferred) and in the case that it is deemed unsafe only one embryo would be transferred.
Transvaginal ultrasound scans are a standard part of the IVF/ICSI treatment cycle and carry no risk to the treatment cycle. Similarly, transvaginal ultrasound scans at 18 and 22 weeks are of no risk to you or your babies.
Blood taking carries the standard risks of a bruise at the site of vein puncture, inflammation of the vein and possible infection, care will be taken to avoid these complications.

III. POSSIBLE ALTERNATIVES:

Participation is voluntary and you may withdraw consent at any stage of the study without your medical care being affected.

Section C

AGREEMENT TO CONSENT

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I understand that the sponsors and investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork Teaching Hospitals and Cork Fertility Centre. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

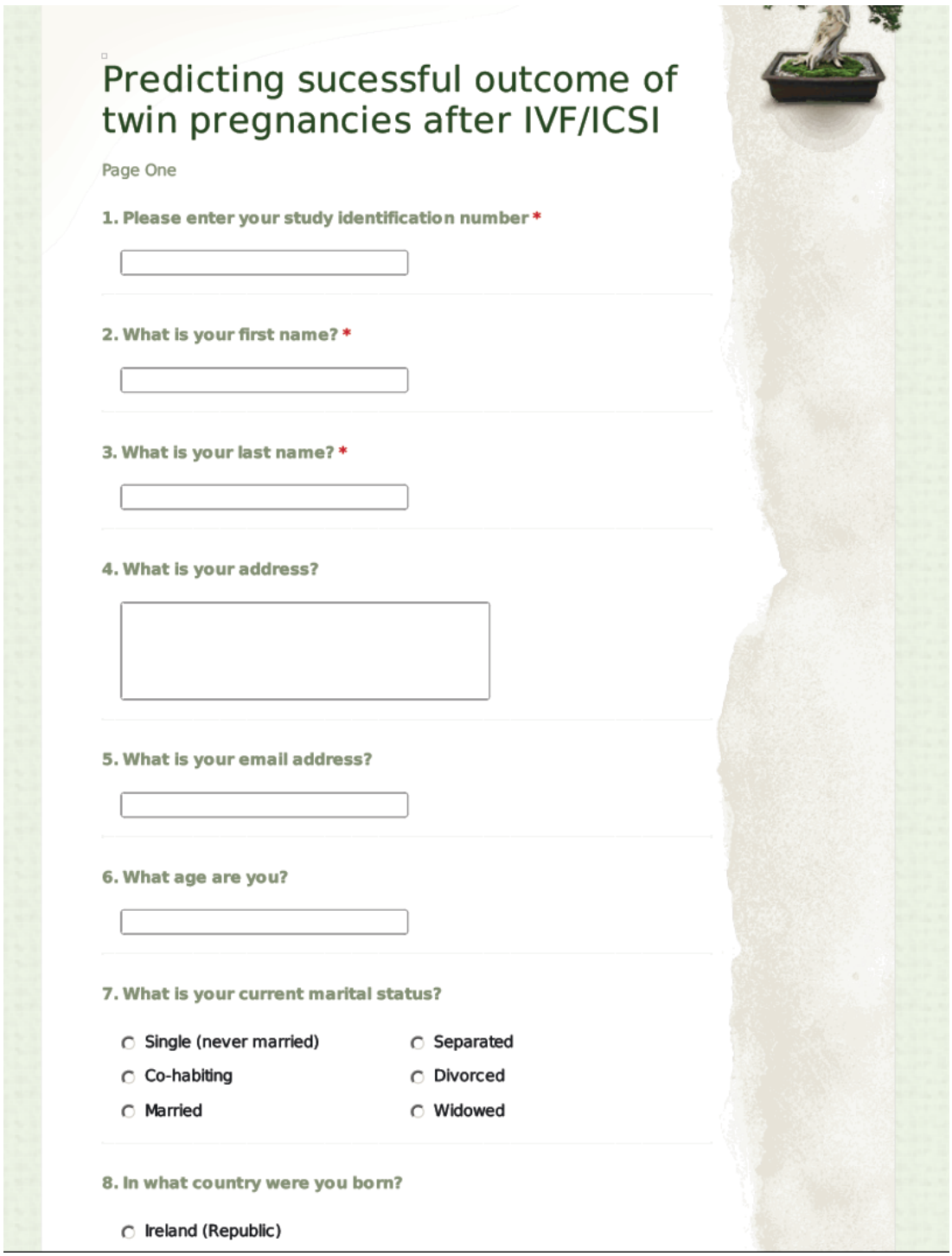
Doctor: _____

Name of Participants:

Signature of Participants:
Witness: _____ Date: _____ Time:

Appendix viii - Sample of survey

Sample of survey distributed to women as part of the survey-prospective study of women attending for fertility consultation or treatment at Cork Fertility Centre and as part of the prospective study of nulliparous women attending for IVF/ICSI treatment at Cork Fertility Centre.



Predicting sucessful outcome of twin pregnancies after IVF/ICSI

Page One

1. Please enter your study identification number *

2. What is your first name? *

3. What is your last name? *

4. What is your address?

5. What is your email address?

6. What age are you?

7. What is your current marital status?

- Single (never married)
- Co-habiting
- Married
- Separated
- Divorced
- Widowed

8. In what country were you born?

- Ireland (Republic)

- Ireland (NI)
- Other UK
- Other, please specify

9. If you were not born in Ireland, in what year did you move to Ireland?

10. What is your ethnic or cultural background?

- White Irish
- Irish Traveller
- Any other white background
- Black or black Irish; African
- Asian or Asian Irish; Chinese
- Other including mixed background

11. What is your weight without clothes?

Stones
or Pounds
or Kilos

12. What is your height without shoes?

Centimetres
or feet and inches

13. How would you describe your general health in the last 12 months?

- Excellent
- Very Good
- Good
- Fair
- Poor
- Very Poor

This section is about you and your household

14. What is the highest level of education you have completed to date?

- ○ Some Primary (not completed)
- ○ Primary or equivalent
- ○ Intermediate/Junior/ Group Certificate or equivalent
- ○ Leaving Cert or equivalent
- ○ Diploma/Certificate
- ○ Primary Degree
- ○ Postgraduate/Higher Degree

15. How would you describe your current employment status?

- ○ Full-time paid work
- ○ Part-time paid work
- ○ Casual paid work
- ○ Looking for first job
- ○ Unemployed
- ○ Student
- ○ Looking after home/family
- ○ Unable to work due to sickness/disability
- ○ Unpaid voluntary work
- ○ Other, please specify

16. How many hours per week do you work (excluding lunch)?

- ○ 0-19
- ○ 20-39
- ○ 40-59
- ○ 60+

17. Do you now find the work place to be a stressful environment?

- ○ Never
- ○ Sometimes
- ○ Often

18. How would you describe your current living accommodation?

- ○ House with a mortgage
- ○ House with no mortgage
- ○ Apartment with a mortgage
- ○ Apartment with no mortgage
- ○ Rented house/apartment (rented privately)
- ○ Rented house/apartment (rented from local authority)
- ○ Caravan/mobile home
- ○ Other

19. Who else lives with you in your household? (Please tick ALL that apply)

- ☐ Your partner/husband
- ☐ Your son/daughter
- ☐ Your mother
- ☐ Your father
- ☐ Your partner's mother
- ☐ Your partner's father
- ☐ Partner's child/children from a previous relationship
- ☐ Your sister(s) and/or brother(s)
- ☐ Your friends(s)
- ☐ No one
- ☐ Nanny/au pair
- ☐ Other

20. What is your approximate level of net household income? This means the total income, after tax and PRSI, of ALL MEMBERS of your household. It includes ALL TYPES of income (e.g. from employment, social welfare payments, child benefit, rents etc.). THE INFORMATION YOU GIVE IS ENTIRELY CONFIDENTIAL AND ONLY FOR USE AS PART OF THIS RESEARCH STUDY. Please tick the box corresponding to the total income range of your household.

- ○ Per week under €193/ Per month under €834/ Per year under €10,000
- ○ Per week €193- €384/ Per month €834-€1,667/ Per year €10,000-€19,999
- ○ Per week €385-€575/ Per month €1,668-€2,500/ Per year €20,000-€29,999

- ○ Per week €576-€767/ Per month €2501-€3,333/ Per year €30,000-€39,999
- ○ Per week €768-€959/ Per month €3,334-€4,167/ Per year €40,000-€49,999
- ○ Per week €960 or more/Per month €4,168 or more/ Per year €50,000 or more

New Page

This section is about your physical activity levels

21. In general, how has your level of physical activity changed since you started trying for a baby?

- ○ Unchanged, my physical activity levels have not changed
- ○ Decreased, I do less physical activity since I started trying for a baby
- ○ Increased, I do more physical activity since I starte trying for a baby

22. Please indicate how many times per week you do the following physical activities. If you do a specific exercise, please indicate the number of mintues on average spent on this exercise each time.

	Number of times per week	Number of minutes per session
Aerobics/gymnastics		
Dancing		
Cycling		
Fast walk		
Leisurely walk		
Jogging, orienteering		
Ball games		
Swimming		
Fitness/health centres		
Badminton		
Tennis		
Horseback riding		
Yoga		

Other (please specify)		

23. On average, how many hours per day do you watch television?

I have no television set
Less than one hour
1 hour
1 1/2 hours
2 hours
2 1/2 hours
3 hours
3 1/2 hours
4 hours
4 1/2 hours
More than 4 hours

24. Is your job physically demanding?

- Yes
- No

25. Do you work with a computer screen?

If no, go to question 26

- Yes
- No

26. How many hours per week do you work with a computer screen?

27. In your job, do you lift between 11-20 kilograms (24-44 pounds) at a time (e.g. heavy boxes?)

- Yes
- No

28. How many times per day do you lift between 11-20kgs (24-44lbs)?

29. How many times per day do you lift more than 20kgs (44lbs)?

New Page

This section is about your diet

For the following questions please note that a cup of coffee/tea/cola refers to a standard mug size (approx. 180mls) or approximately half a can of cola.

30. Do you drink coffee?

If no, go to question 31.

- ○ No, I never drink coffee
- ○ Yes, but I stopped when I started trying for a baby
- ○ Yes, and I continue to drink coffee since I started trying for a baby

31. How many cups of coffee do you drink per day?

- ○ 1 to 3 cups
- ○ 4 to 6 cups
- ○ 7 or more cups

32. Do you drink tea?

If no, go to question 33.

- ○ No, I never drink tea
- ○ Yes, but I stopped when I started trying for a baby
- ○ Yes, and I continue to drink tea since I started trying for a baby

33. How many cups of tea do you drink per day?

- ○ 1 to 3 cups of tea
- ○ 4 to 6 cups of tea
- ○ 7 or more cups of tea

34. Do you drink cola/high energy drinks (e.g. Coca-Cola, Red Bull)?

If no, go to question 35.

- ○ No, I never drink cola/high energy drinks
- ○ Yes, but I stopped drinking cola/high energy drinks since I started trying for a baby
- ○ Yes, and I continue to drink cola/high energy drinks since I started trying for a baby

35. Thinking about the food you usually eat, how often do you eat fruit (including fresh, frozen, dried, tinned and pure fruit juice)?

- ○ More than once per day
- ○ Once every day
- ○ Most days
- ○ Once or twice per week
- ○ Less than once per week

36. Thinking about the food you usually eat, how often do you eat vegetable (including salad, fresh, frozen, dried, tinned vegetables but excluding potatoes)?

- ○ More than once per day
- ○ Once per day
- ○ Most days
- ○ Once or twice per week
- ○ Less that once per week

New Page

This question is about your drinking and smoking behaviours

37. About alcohol, which of the following best describes your behaviours since you started trying for a baby

- ○ I drink regularly, about the same as before I started trying for a baby
- ○ I drink regularly, but I cut down since I started trying for a baby
- ○ I drink more alcohol since I started trying for a baby
- ○ I drink once in a while
- ○ I stopped drinking when I started trying for a baby
- ○ I used to drink alcohol but I stopped before I started trying for a baby
- ○ I have never drunk alcohol

Please use the following as a guide to help you determine how many drinks you have. A drink is
- A half pint/glass of beer
- A single measure of spirits
- A single glass of wine, sherry or port
- A bottle of alcopops

38. If you drink alcohol every day, how many units do you usually drink per day, since you started trying for a baby?

39. How many units of alcohol do you drink per week since you started trying for a baby?

40. How many DAYS per week would you drink five or more units or alcohol?

(Please write 0 if none and write 7, if you drink more than 5 units every day of the week etc.)

41. About cigarettes, which of the following best describes you?

- I smoke regularly now, about the same as I did before I started trying for a baby
- I smoke regularly now, but I cut down when I started trying for a baby
- I smoke more now than I did before I started trying for a baby
- I smoke once in a while
- I stopped smoking when I started trying for a baby
- I used to smoke but stopped before I started trying for a baby and I don't smoke cigarettes now
- I have never smoked cigarettes

42. If you smoke cigarettes every day, how many do you smoke?

43. If you smoke cigarettes less often than every day, how many do you usually smoke per week?

44. Do you use drugs?

- ○ No, I do not use drugs
- ○ Yes, but I stopped using drugs when I started trying for a baby
- ○ Yes, and I continue to use drugs since I started trying for a baby

45. If yes, which of the following drugs did you use before trying for a baby?

- ☐ Marijuana (grass/pot) or cannabis (hash, hash oil)
- ☐ Tranquilisers or sedatives (Barbs, Downers, Jellie) without a doctor's prescription
- ☐ Tranquilisers or sedatives with a doctor's prescription (e.g. Benzodiazepines)
- ☐ Methadone without a doctor's prescription
- ☐ Methadone with a doctor's prescription
- ☐ Cocaine (crack, coke)
- ☐ Heroin (smack, skag)
- ☐ Ecstasy (E, XTC) Amphetamine (Speed, Whizz) or LSD (Acid, Trips)
- ☐ Other

46. If you use/used drugs while trying for a baby, which of the follwoing do you or did you use?

- ☐ Marijuana
- ☐ Tranqulisers without a doctor's prescription
- ☐ Tranquilisers with a doctor's prescription
- ☐ Methadone without a doctor's prescription
- ☐ Methadone with a doctor's prescription
- ☐ Cocaine (crack, coke)
- ☐ Heroin (smack, skag)
- ☐ Ecstasy (E, XTC), Amphetamine (Speed, Whizz), LSD (Acid, Trips)

☐ Other

New Page

This section is about how you feel

47. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks....

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
Did you feel full of life?	○	○	○	○	○	○
Have you been a very nervous person?	○	○	○	○	○	○
Have you felr so down in the dumps that nothing could cheer you up?	○	○	○	○	○	○
Have you felt calm and peaceful?	○	○	○	○	○	○
Did you have a lot of energy?	○	○	○	○	○	○
Have you felt downhearted and blue?	○	○	○	○	○	○
Did you feel worn out?	○	○	○	○	○	○
Have you been a happy person?	○	○	○	○	○	○
Did you feel tired?	○	○	○	○	○	○

48. For each of the following statements, please tick one box which shows how you feel about the support you have right now.

	Always	Most of the time	Some of the time	Rarely	Never
I have good friends who support me	○	○	○	○	○
My family is always there for me	○	○	○	○	○
My husband/partner helps me a lot	○	○	○	○	○
There is conflict with my husband/partner	○	○	○	○	○
I feel controlled by my husband/partner	○	○	○	○	○
I feel loved by my husband/partner	○	○	○	○	○

49. The following statements ask about your feelings and thoughts in the LAST MONTH. In each case, indicate, by ticking the appropriate box, how often you felt or thought a certain way.
Although some questions are similar, there are differences between them and you should treat each on as a separate question.

	Very often	Fairly often	Sometimes	Almost never	Never
In the past month, how often have you felt that you were unable to control the important things in your life?	○	○	○	○	○
In the past month, how often have you felt confident about your ability to handle personal problems?	○	○	○	○	○
In the past month, how often have you felt that things were going your way?	○	○	○	○	○
In the past month, how often have you felt difficulties were piling up so high that you could not overcome them?	○	○	○	○	○

50. Please rate your agreement by ticking the appropriate response. Be as honest and as accurate as you can throughout, and try not to let your response to one statement influence your response to other statements. There are no "correct" or "incorrect" answers. Answer according to your own feelings.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
In uncertain times, I usually expect the best.	○	○	○	○	○
Generally, it's easy for me to relax.	○	○	○	○	○
If something can go wrong, for me it will.	○	○	○	○	○
Generally, I am optimistic about my future.	○	○	○	○	○
Generally, I expect things to go my way.	○	○	○	○	○
Generally, I count on good things happening to me.	○	○	○	○	○
Generally, I expect more good things to happen to me than	○	○	○	○	○

Miscarriage	○	○	○
Other stressful, traumatic event	○	○	○

New Page

This section is about your general health and medications

54. Have you previously been diagnosed by a doctor with a medical illness?

- ○ Yes
- ○ No

55. Have you ever been diagnosed by a doctor with any of the following conditions?

	Yes	No	Attended Dr. for this condition, in last 6 months, Yes?	Attended Dr. for this condition in last 6 months, No?
Asthma	☐	☐	☐	☐
Chronic bronchitis, COPD, emphysema	☐	☐	☐	☐
Angina	☐	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐	☐
Osteoarthritis	☐	☐	☐	☐
Lower back pain or other chronic back condition	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐
Cancer	☐	☐	☐	☐
Urinary incontinence, problems controlling the bladder	☐	☐	☐	☐
Anxiety	☐	☐	☐	☐
Depression	☐	☐	☐	☐
Other, please specify below	☐	☐	☐	☐
None of the above	☐	☐	☐	☐
	☐	☐	☐	☐

56. Do you take regular medications for any of the following conditions?

	Please tick all that apply
Angina	☐

Antiarrhythmic	☐
Blood pressure	☐
Aspirin	☐
Cardiac	☐
Cholesterol	☐
Nitrates	☐
Diabetes	☐
Diuretics/water tablets	☐
Plavix	☐
Warfarin	☐
Anxiety	☐
Depression	☐
Psychosis	☐
Arthritis	☐
Respiratory	☐
Osteoporosis	☐
Parkinsons disease	☐
Thyroid	☐
Ulcer	☐
Vitamins	☐
Heparin	☐
Progesterone	☐
Pain killers	☐
Steroids	☐
Anti-nausea	☐
Gout	☐
HRT	☐
Night sedations	☐
Other, please specify	☐

57. Did you stop taking any of these medications since you started trying for a baby? If yes please specify which one(s)

○ Yes

○ No

Comments

58. Have you taken any of these short-terms/new medications in the past 6 months?

- [ ] None
- [ ] Antibiotics
- [ ] Cold medications
- [ ] Anti-inflammatory medications (e.g.ibuprofen, neurofen)
- [ ] Other

59. Have you taken vitamin, mineral or other herbal supplements since you started trying for a baby?

	Please tick all that apply
Folic acid	☐
Iron	☐
Multivitamins	☐
Essential Fatty Acids	☐
Prebiotics/Probiotics (e.g. acidobacillus, lactobacillus)	☐
Herbal supplements (please state name below)	☐
Other (please state below)	☐

Comments

New Page

This section is about your reproductive health

60. Approximately how old were you when you had your first period?

61. How often do you get a period? (i.e. your cycle length in days, counting from the first day of your period until the first day of your next period)

62. If you have been pregnant previously, what is the interval between that pregnancy and now? (If you have never been pregnant, please go to question 69)

- ○ Less than 12 months
- ○ 12-18 months
- ○ 18-24 months
- ○ Greater than 24 months

63. Have you ever had a miscarriage?

- ○ Never
- ○ Yes, once
- ○ Yes, twice
- ○ Yes, three or more times

64. Using the table below please indicate how your miscarriage(s) was (were) managed

	Conservatively (managed at home, no intervention)	Medical management (tablets)	Surgically (operation, D+C/ERPC)
First	☐	☐	☐
Second	☐	☐	☐
Third	☐	☐	☐
Fourth	☐	☐	☐
Fifth	☐	☐	☐
Sixth	☐	☐	☐

65. Was your most recent pregnancy achieved through fertility treatment?

- ○ No, conceived spontaneously
- ○ Yes, drugs only (e.g. Clomid)
- ○ Yes, IUI (Intrauterine Insemination)
- ○ Yes, IVF (In-Vitro Fertilisation)
- ○ Yes, ICSI (Intra-Cytoplasmic Sperm Injection)
- ○ Yes, with donor egg/donor sperm

66. If yes, please specify the fertility issue(s). Please tick all that apply

- ☐ Ovulation
- ☐ Tubal
- ☐ Endometriosis

- [ ] Other female diagnosis (specify below)
- [ ] Poor sperm quality/count
- [ ] Other male diagnosis (specify below)
- [ ] Unexplained
- [ ] Don't know/don't remember

Comments

67. Regarding sexual intercourse in early pregnancy, please tick the level of bleeding you experienced (if applicable)

- ○ Never
- ○ Rarely
- ○ Occasionally
- ○ Often
- ○ Every time

68. Did you have nausea and vomiting in your most recent pregnancy?

- ○ None
- ○ Mild
- ○ Moderate
- ○ Severe (needing to come to hospital)

69. How may days per week did you have nausea and vomiting?

70. How long have you been trying for a baby?

- ○ Less than 12 months
- ○ 12- 18 months
- ○ 18-24 months
- ○ Greater than 24 months

71. Has anyone in your family been affected by pre-eclampsia?

- Yes
- No

72. If yes, please specify which family members and relatives (of female partner only)

- [ ] Grandmother(s)
- [ ] Mother
- [ ] Aunt(s)
- [ ] Sister(s)
- [ ] Cousin(s)

73. Has anyone in your family (mother/sister(s)) had a pre-term labour (delivered a baby at less than 37 weeks of pregnancy)? (tick all that apply)

- [ ] No family history of preterm labour
- [ ] Yes, mother
- [ ] Yes, one sister
- [ ] Yes, more than one sister

74. Has anyone in your family (mother/sister) a history of genital prolapse?

- [ ] No, no family history of genital prolapse
- [ ] Yes, my mother
- [ ] Yes, one sister
- [ ] Yes, more than one sister

New Page

Final Section- This section is about your partner's characteristics

75. Are you planning to use donor sperm?

- Yes
- No

76. How would you describe your partner's current employment status?

- Full-time paid work

- ○ Part-time paid work
- ○ Casual paid work
- ○ Looking for first job
- ○ Unemployed
- ○ Student or pupil
- ○ Looking after home/family
- ○ Unable to work due to sickness/disability
- ○ Unpaid voluntary work
- ○ Other

77. Has you partner been diagnosed by a doctor with any of the following conditions?

- ☐ Asthma
- ☐ Chronic bronchitis, COPD, emphysema
- ☐ Angina
- ☐ Rheumatoid arthritis
- ☐ Osteoarthritis
- ☐ Lower back pain or other chronic back condition
- ☐ Diabetes
- ☐ Cancer
- ☐ Urinary incontinence, problems in controlling the bladder
- ☐ Anxiety
- ☐ Depression
- ☐ Other

78. Does your partner drink alcohol?

- ○ No, he never drinks alcohol
- ○ Yes, he drinks alcohol
- ○ I don't know

79. If your partner drinks alcohol every day, how many units does he drink (PER DAY) since you started trying for a baby?

80. If your partner drinks alcohol less often than every day, how many units of alcohol does he drink (PER WEEK) since you started

trying for a baby?

81. How many DAYS per week would your partner drink 5 or more units of alcohol? (Please write 0 if none and 7 if he drinks more than 5 units of alcohol every day etc.)

82. Does your partner smoke?

- ○ No, he never smokes
- ○ Yes, but he stopped when we started trying for a baby
- ○ Yes and he continues to smoke since we started trying for a baby

83. If your partner smokes cigarettes every day, how many does he usually smoke? (Please indicate the number per day)

84. If he smokes cigarettes less often than every day, how many cigarettes does he smoke per week? (Please indicate number per week)

85. Which statement best describes your partner's smoking habits since you started trying for a baby?

- ○ He never smokes around me
- ○ He occasionally smokes around me
- ○ He usually smokes around me

86. If your partner uses drugs, which of the following drugs has he used since you sterted trying for a baby?

- ○ My partner does not use drugs
- ○ I don't know
- ○ Marijuana (grass, pot) or cannabis (hash)
- ○ Tranquilisers or sedatives without a doctor's prescription
- ○ Tranquilisers or sedatives with a doctor's prescription
- ○ Methadone without a doctor's prescription

trying for a baby?

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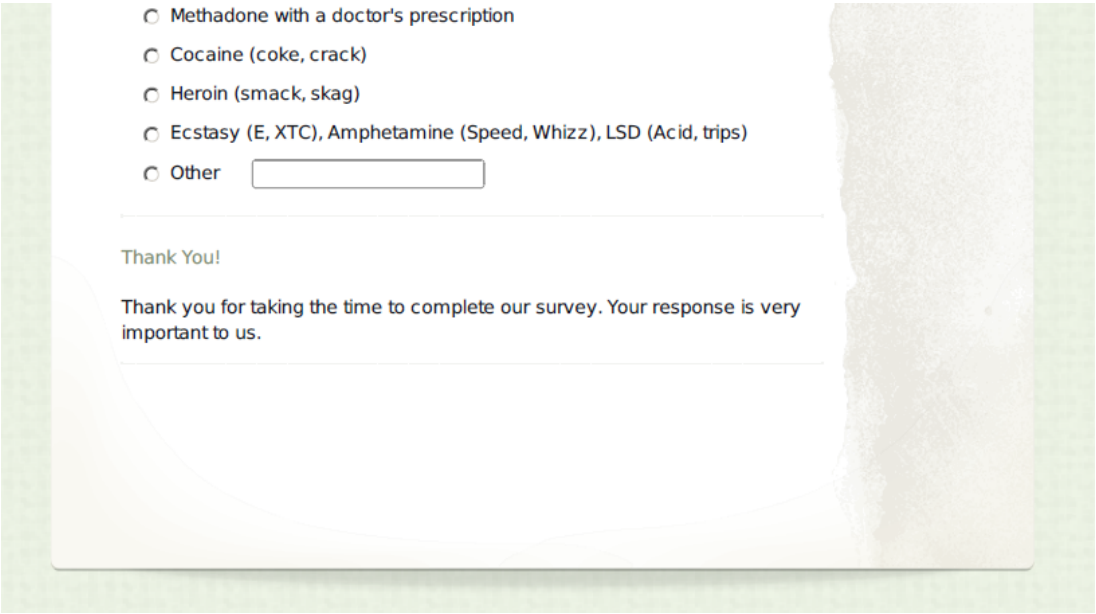
- ○ Tranquilisers or sedatives without a doctor's prescription
- ○ Tranquilisers or sedatives with a doctor's prescription
- ○ Methadone without a doctor's prescription


Methadone with a doctor's prescription
Cocaine (coke, crack)
Heroin (smack, skag)
Ecstasy (E, XTC), Amphetamine (Speed, Whizz), LSD (Acid, trips)
Other
Thank You!
Thank you for taking the time to complete our survey. Your response is very important to us.